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Borenstein et al.

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(54) **SYSTEMS AND METHODS FOR CELL TRANSDUCTION**

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(22) Filed: **Jun. 5, 2017**

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Related U.S. Application Data

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(51) **Int. Cl.**

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C12N 15/87	(2006.01)
C12M 1/00	(2006.01)
C12M 1/12	(2006.01)
C12N 5/0783	(2010.01)
B01L 3/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/87*** (2013.01); ***B01L 3/502746*** (2013.01); ***C12M 23/40*** (2013.01); ***C12M 25/00*** (2013.01); ***C12M 27/18*** (2013.01); ***C12M 29/04*** (2013.01); ***C12M 35/08*** (2013.01); ***C12N 5/0636*** (2013.01); *B01L 3/502761* (2013.01); *B01L 2200/0668* (2013.01); *B01L 2300/087* (2013.01); *B01L 2300/0877* (2013.01); *B01L 2400/0487* (2013.01); *B01L 2400/086* (2013.01); *C12N 2510/00* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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Primary Examiner — James S Ketter

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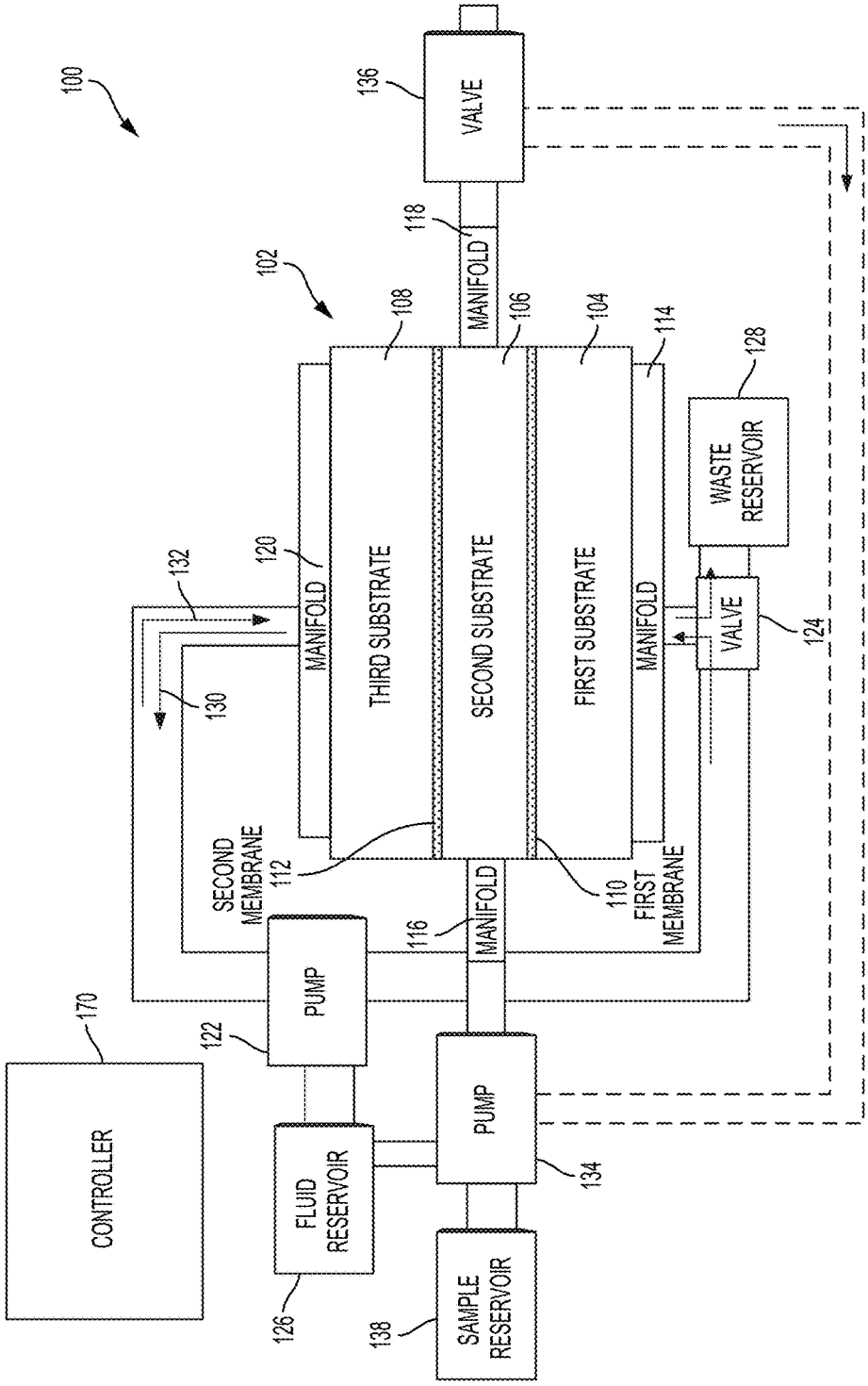
(57) **ABSTRACT**

Systems and methods are disclosed herein for use in transducing, activating, and otherwise treating cells. Cells are introduced into an inner layer of a multi-layered stack that defines at least one flow chamber and a plurality of cell entrainment regions. Vertical flow through the stack entrains the cells in the cell entrainment regions along with genetic information introduction agents or other additives, before the cells are washed using a reverse vertical flow and are collected from the device.

23 Claims, 22 Drawing Sheets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FIRST SUBSTRATE
MANIFOLD
VALVE
124
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SECOND MEMBRANE
112
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FIRST MEMBRANE
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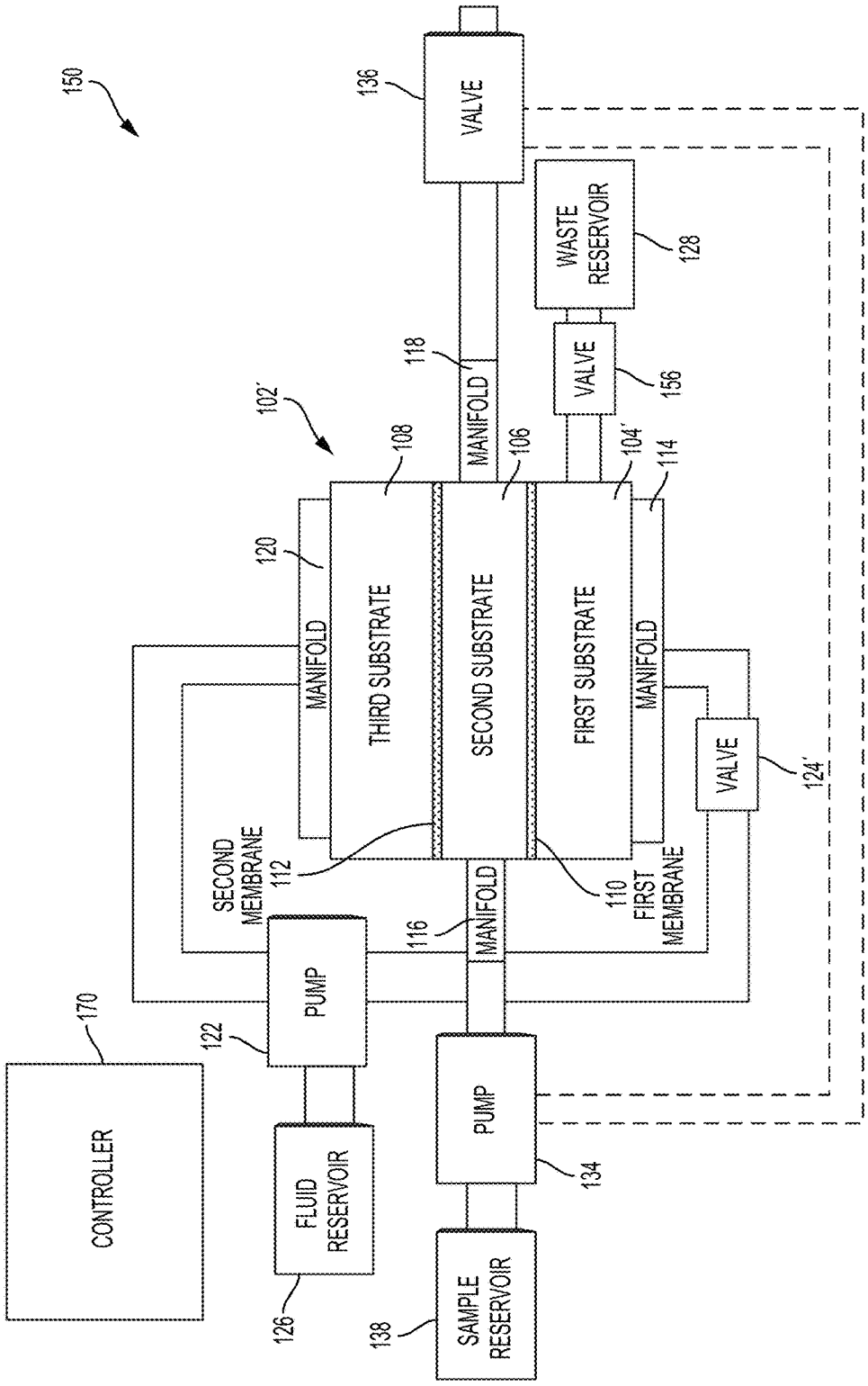
SAMPLE RESERVOIR

FIG. 1A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THIRD SUBSTRATE
SECOND SUBSTRATE
FIRST SUBSTRATE
MANIFOLD
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112
110
FIRST MEMBRANE
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138

FIG. 1B

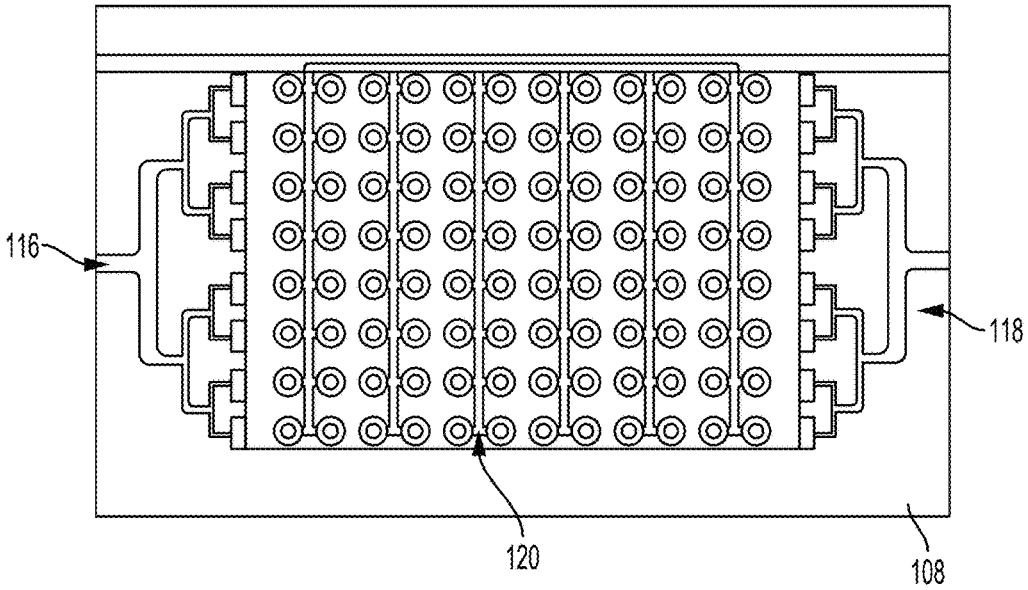


FIG. 1C

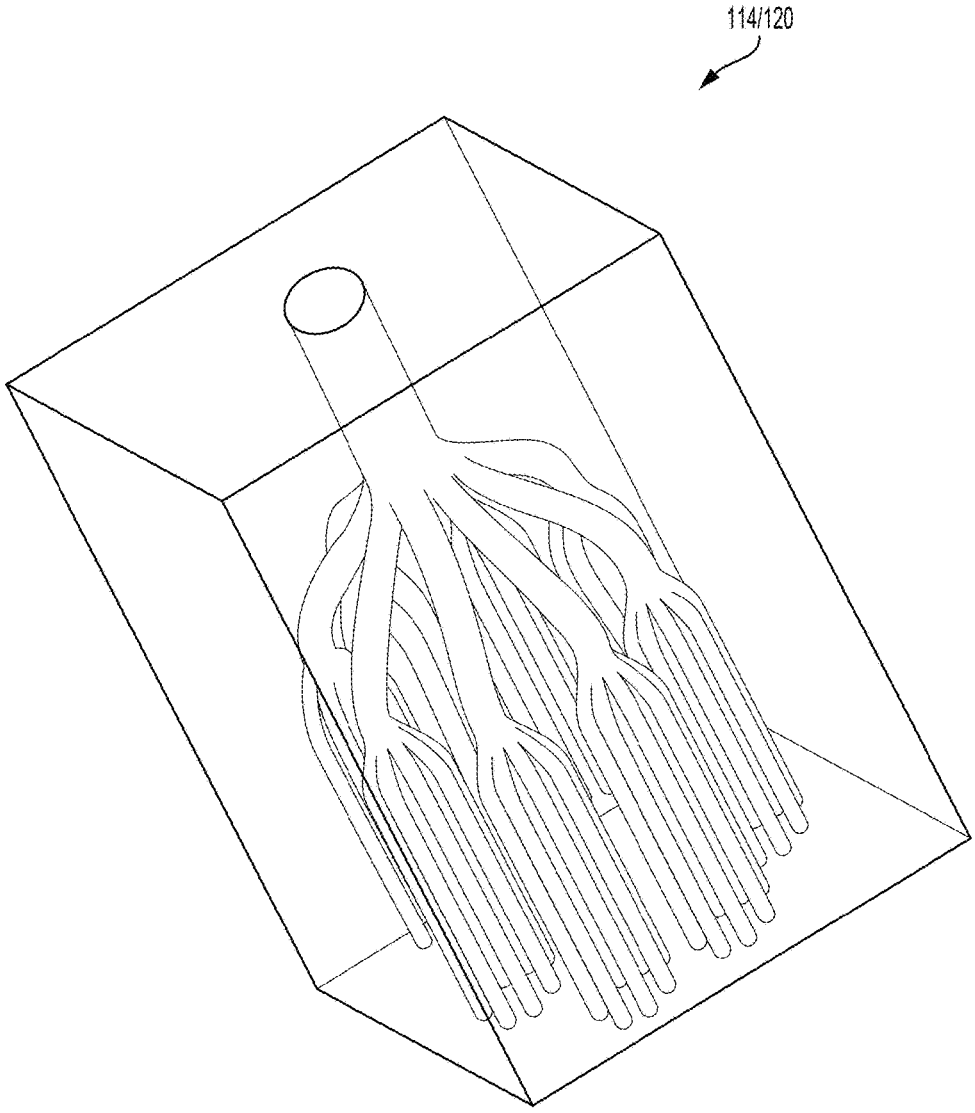


FIG. 1D

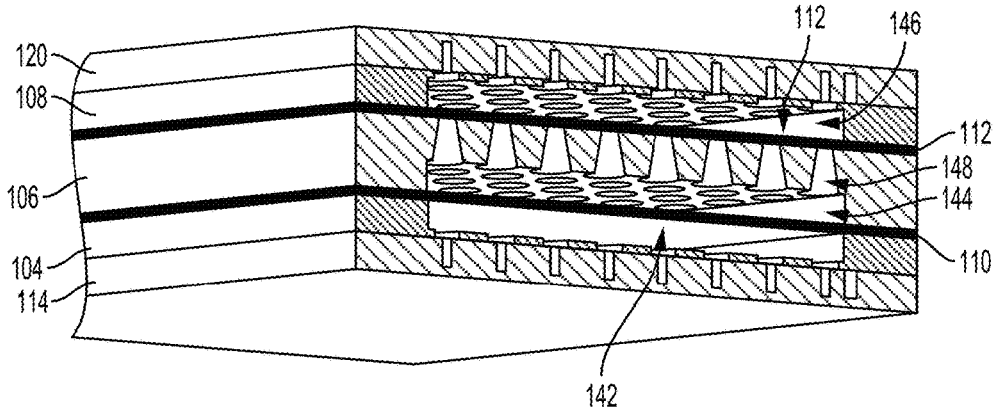


FIG. 1E

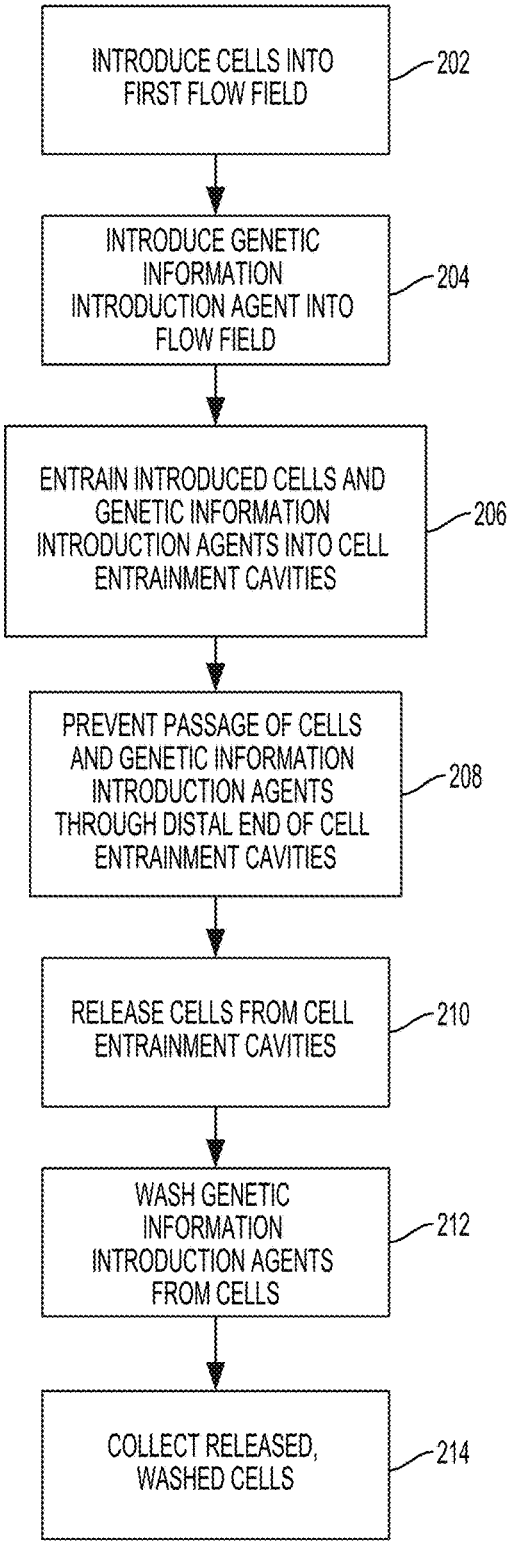

INTRODUCE CELLS INTO FIRST FLOW FIELD
202
INTRODUCE GENETIC INFORMATION INTRODUCTION AGENT INTO FLOW FIELD
204
ENTRAIN INTRODUCED CELLS AND GENETIC INFORMATION INTRODUCTION AGENTS INTO CELL ENTRAINMENT CAVITIES
206
PREVENT PASSAGE OF CELLS AND GENETIC INFORMATION INTRODUCTION AGENTS THROUGH DISTAL END OF CELL ENTRAINMENT CAVITIES
208
RELEASE CELLS FROM CELL ENTRAINMENT CAVITIES
210
WASH GENETIC INFORMATION INTRODUCTION AGENTS FROM CELLS
212
COLLECT RELEASED, WASHED CELLS
214

FIG. 2

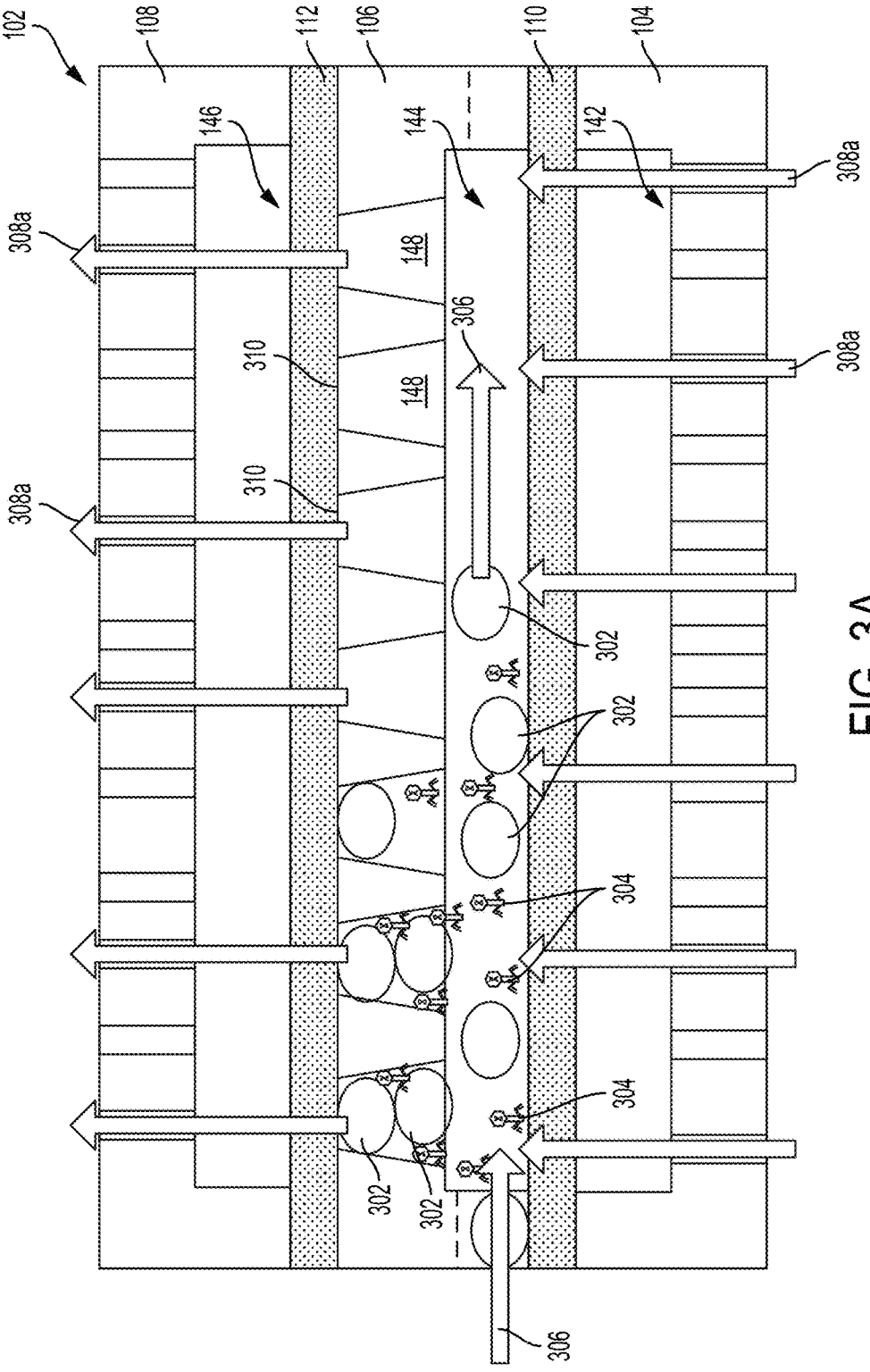


FIG. 3A

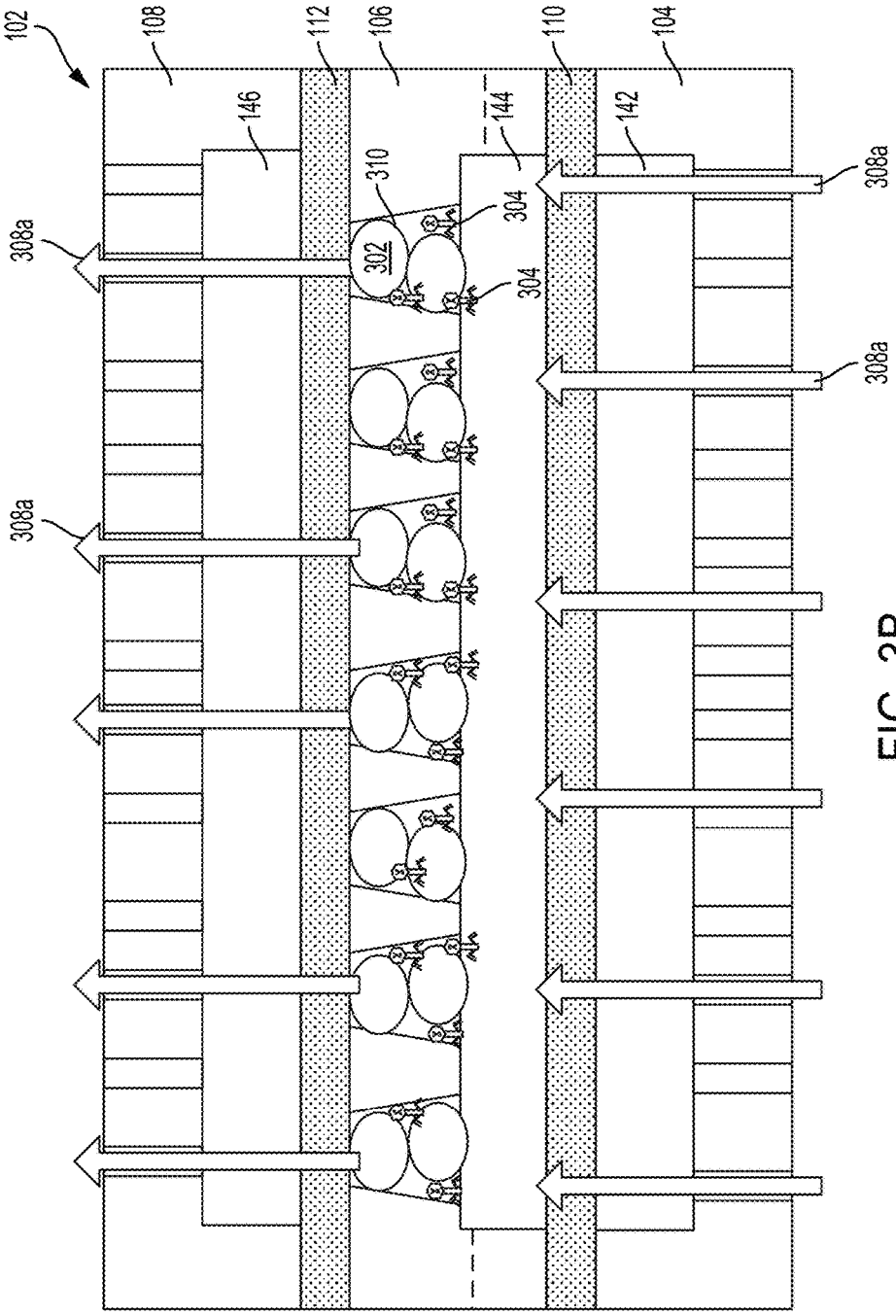


FIG. 3B

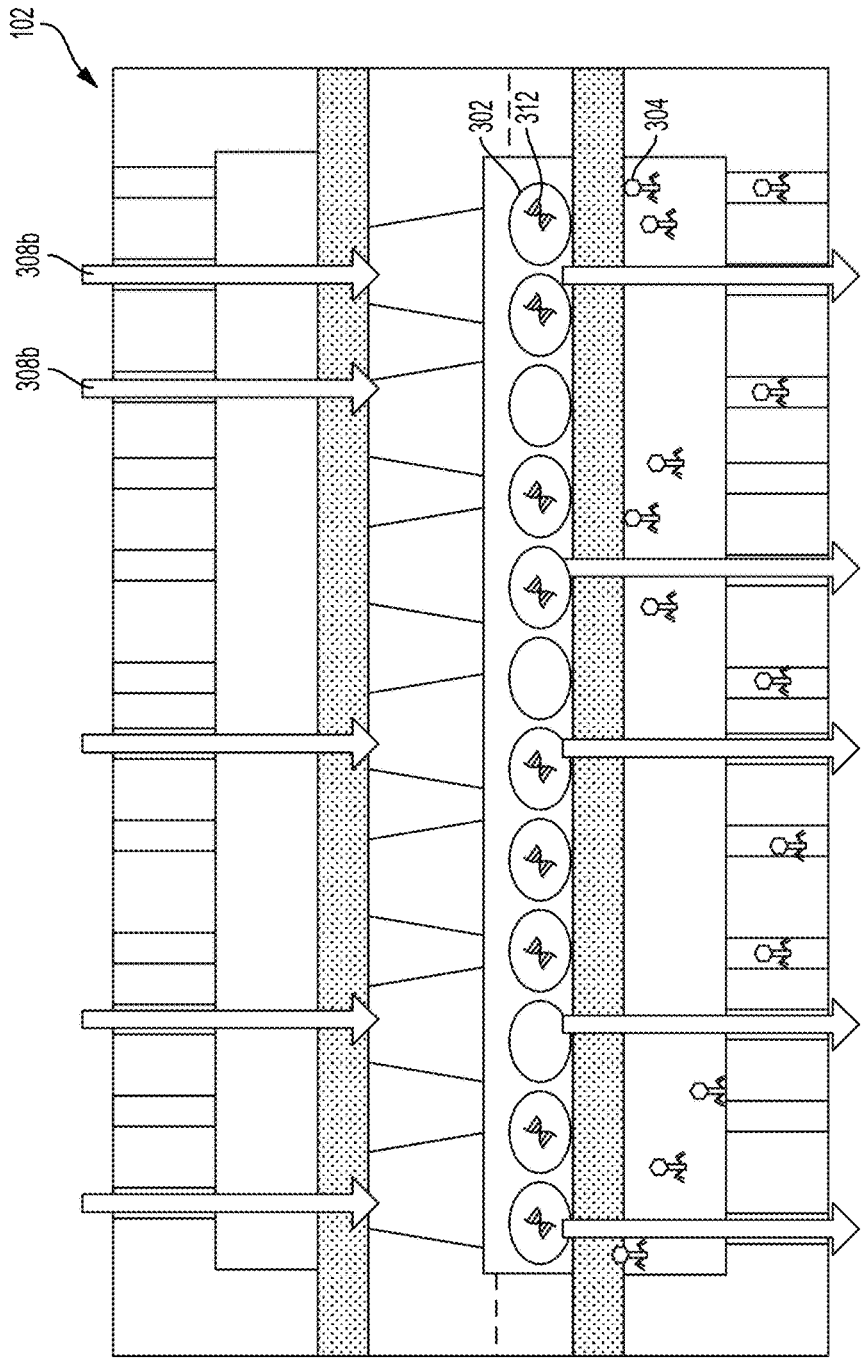


FIG. 3C

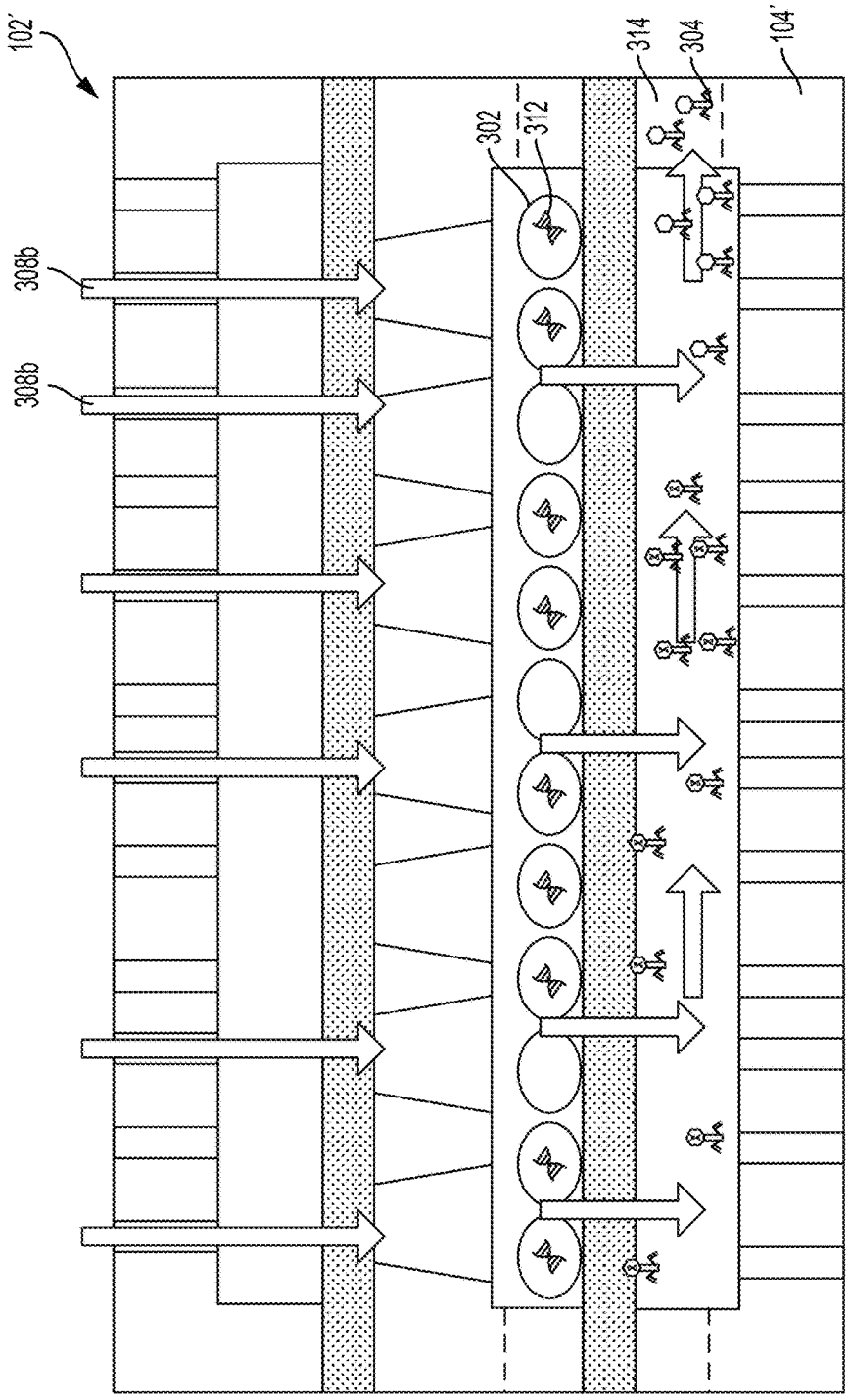


FIG. 3D

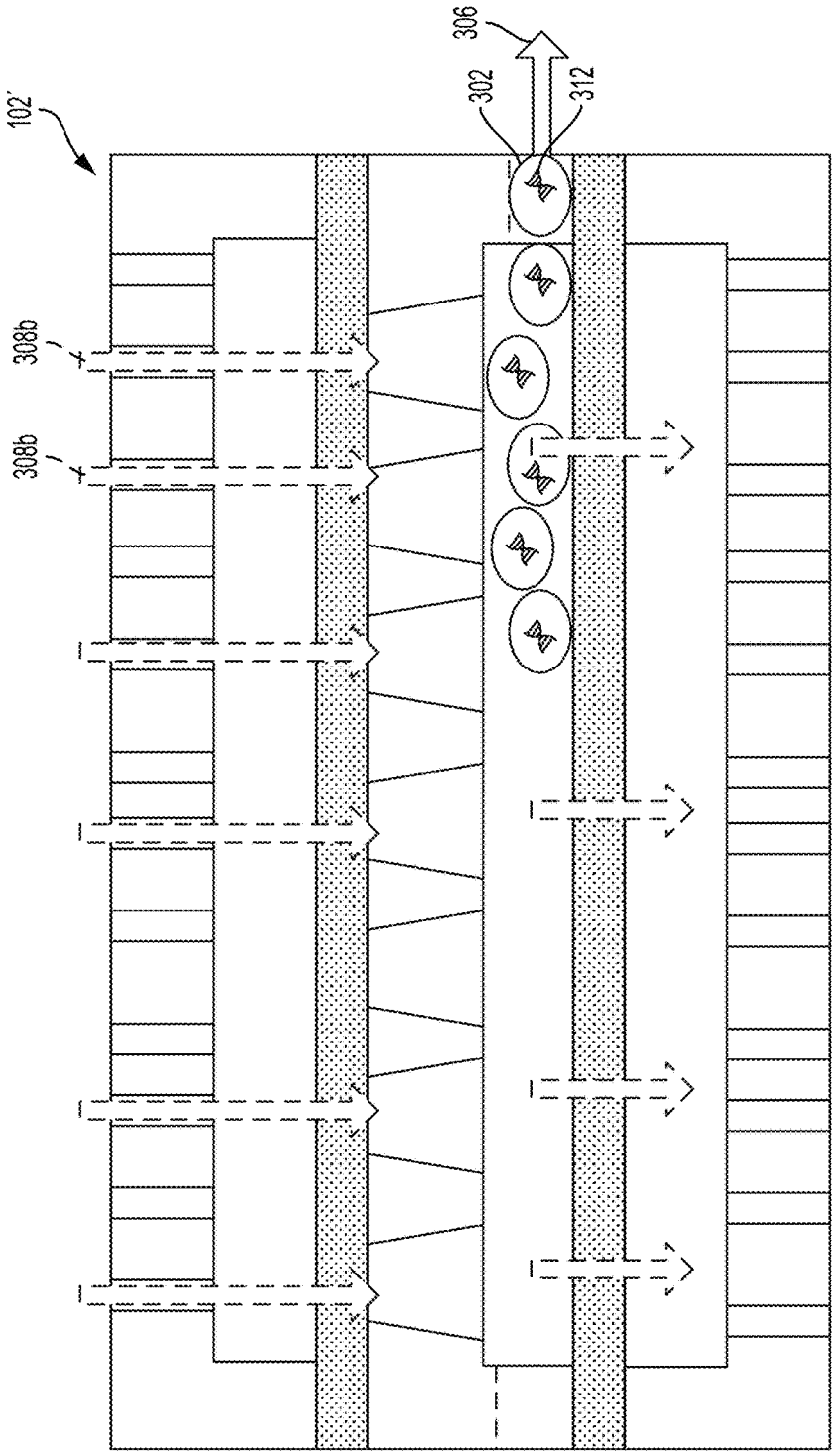


FIG. 3E

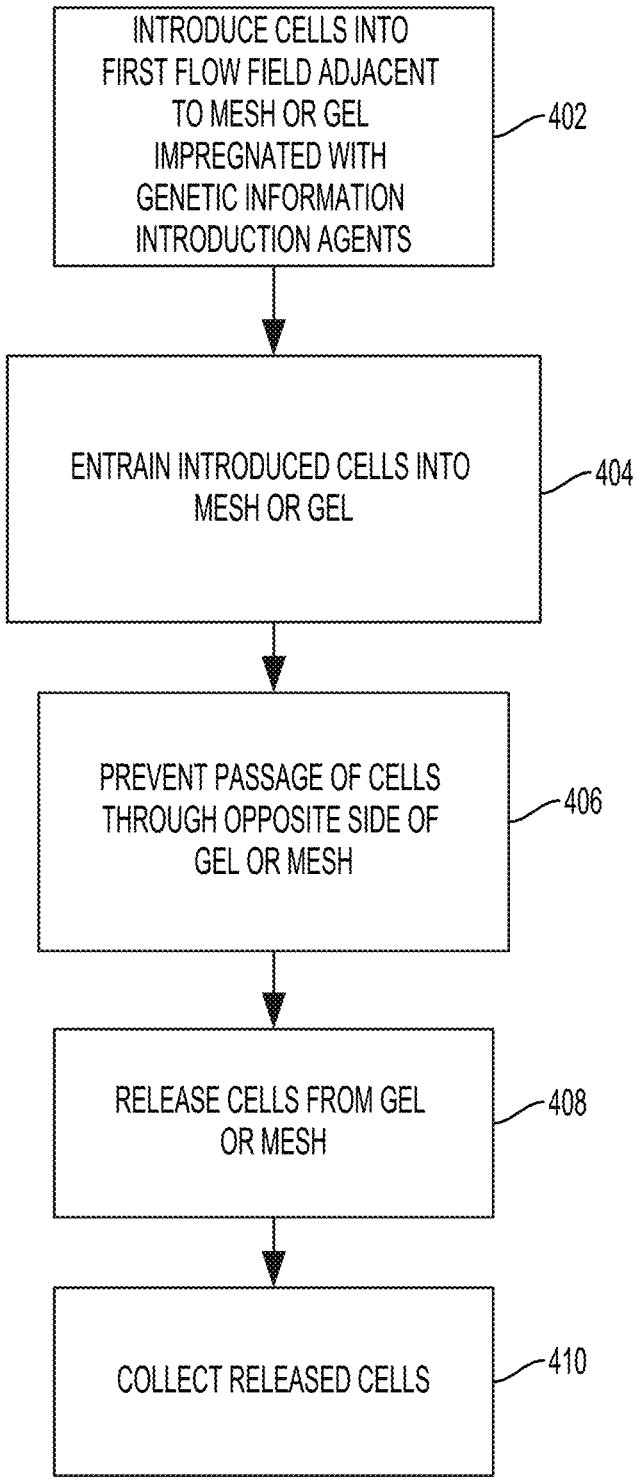

INTRODUCE CELLS INTO FIRST FLOW FIELD ADJACENT TO MESH OR GEL IMPREGNATED WITH GENETIC INFORMATION INTRODUCTION AGENTS
402
ENTRAIN INTRODUCED CELLS INTO MESH OR GEL
404
PREVENT PASSAGE OF CELLS THROUGH OPPOSITE SIDE OF GEL OR MESH
406
RELEASE CELLS FROM GEL OR MESH
408
COLLECT RELEASED CELLS
410

FIG. 4

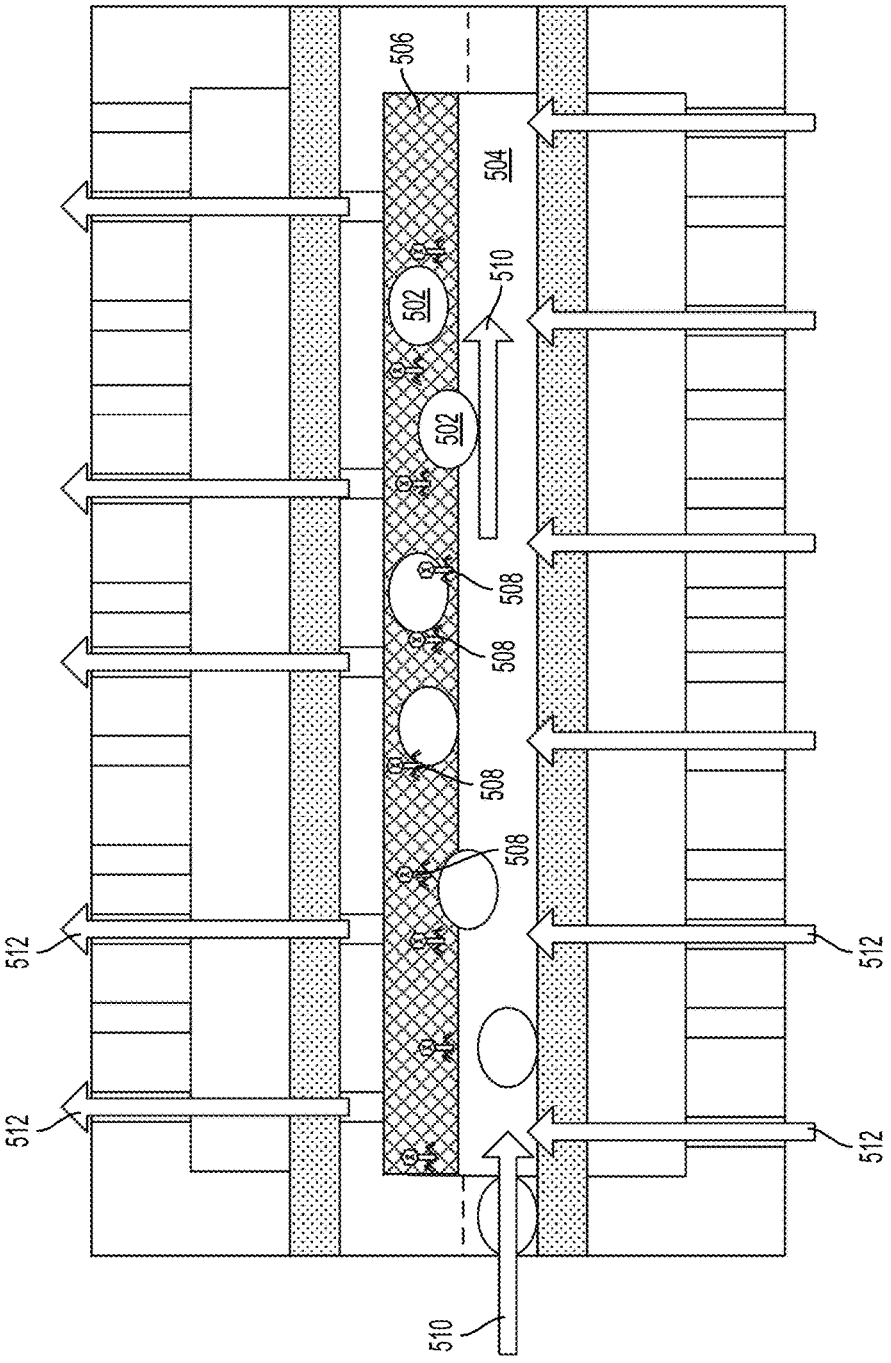


FIG. 5A

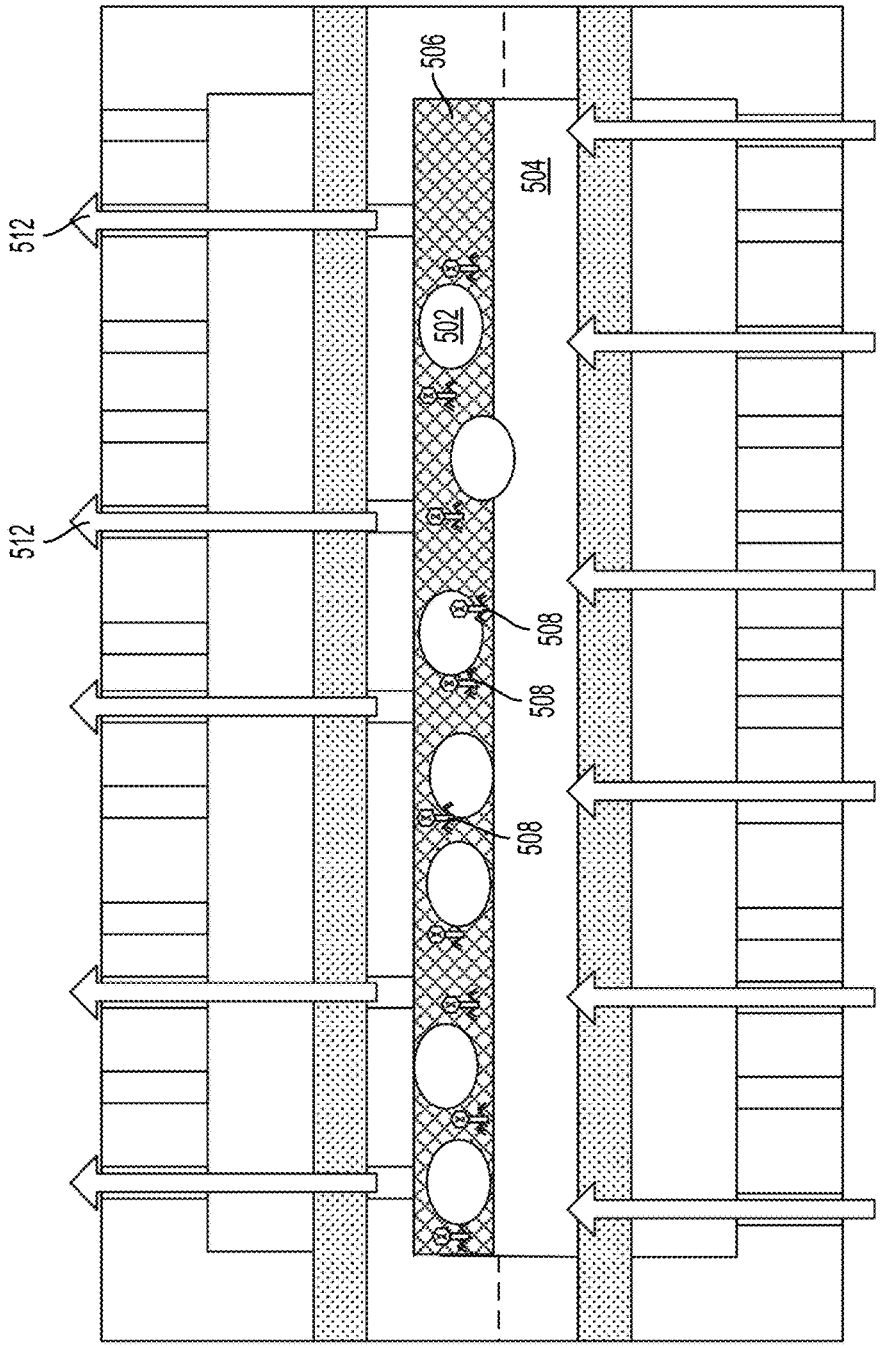


FIG. 5B

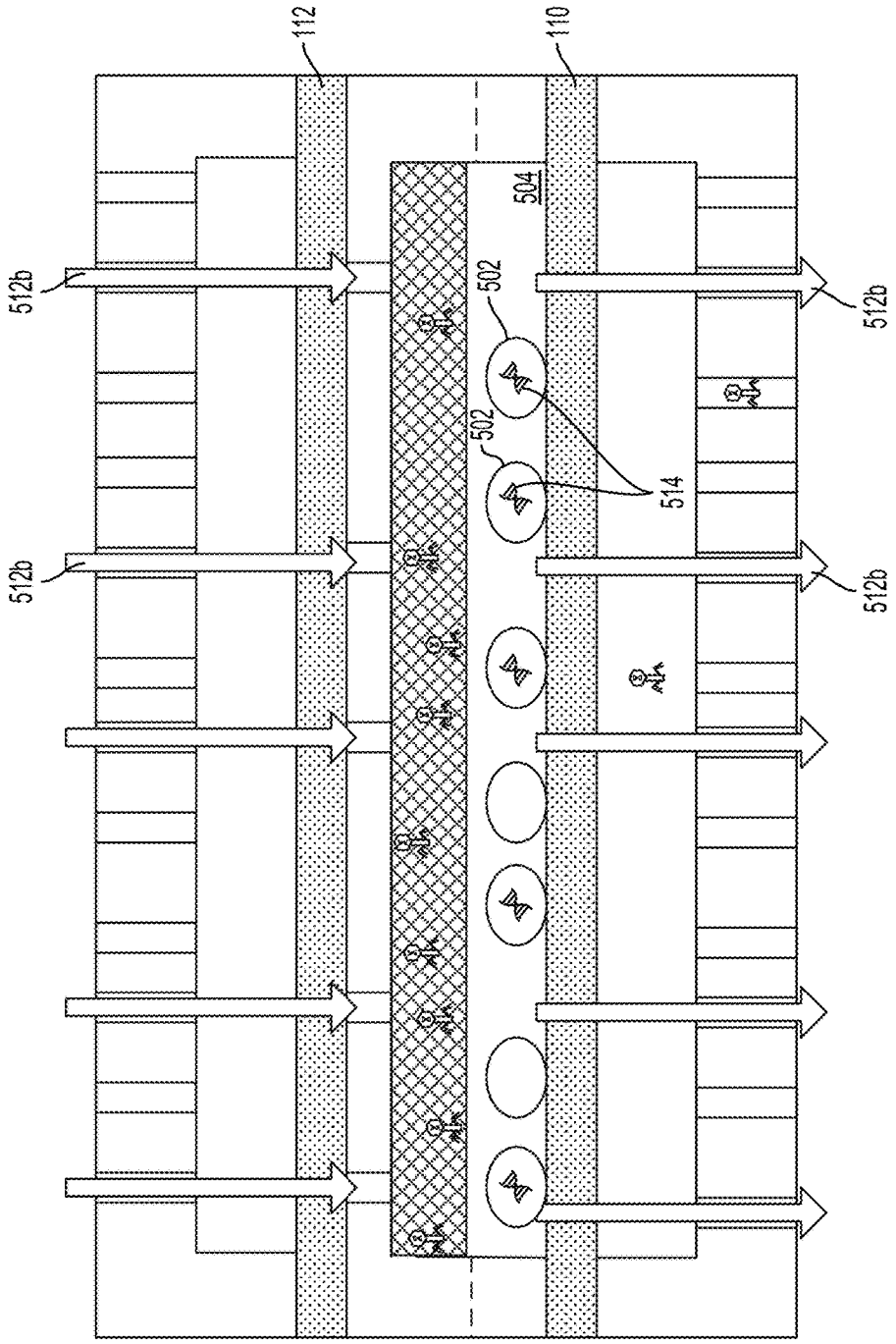


FIG. 5C

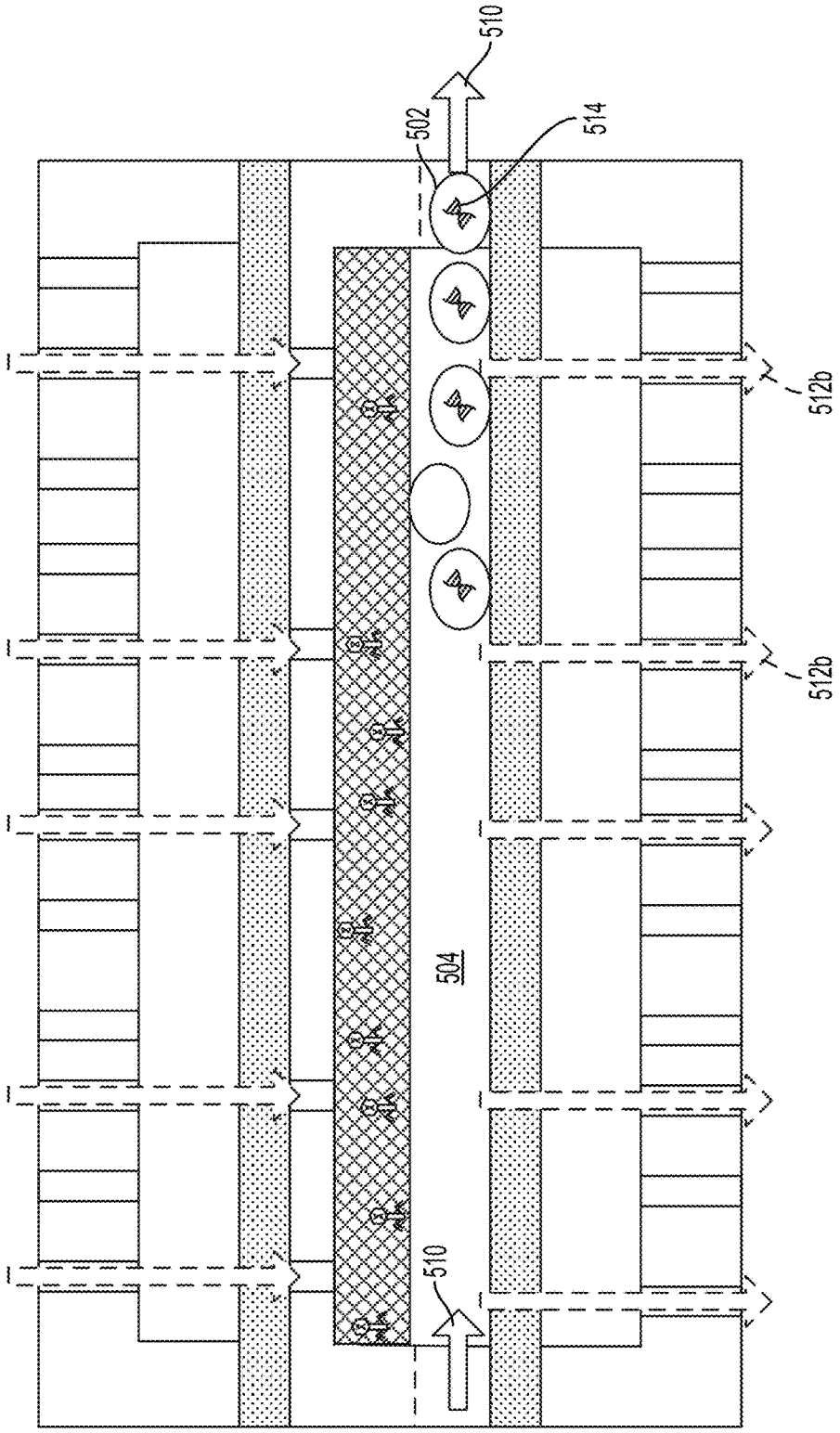


FIG. 5D

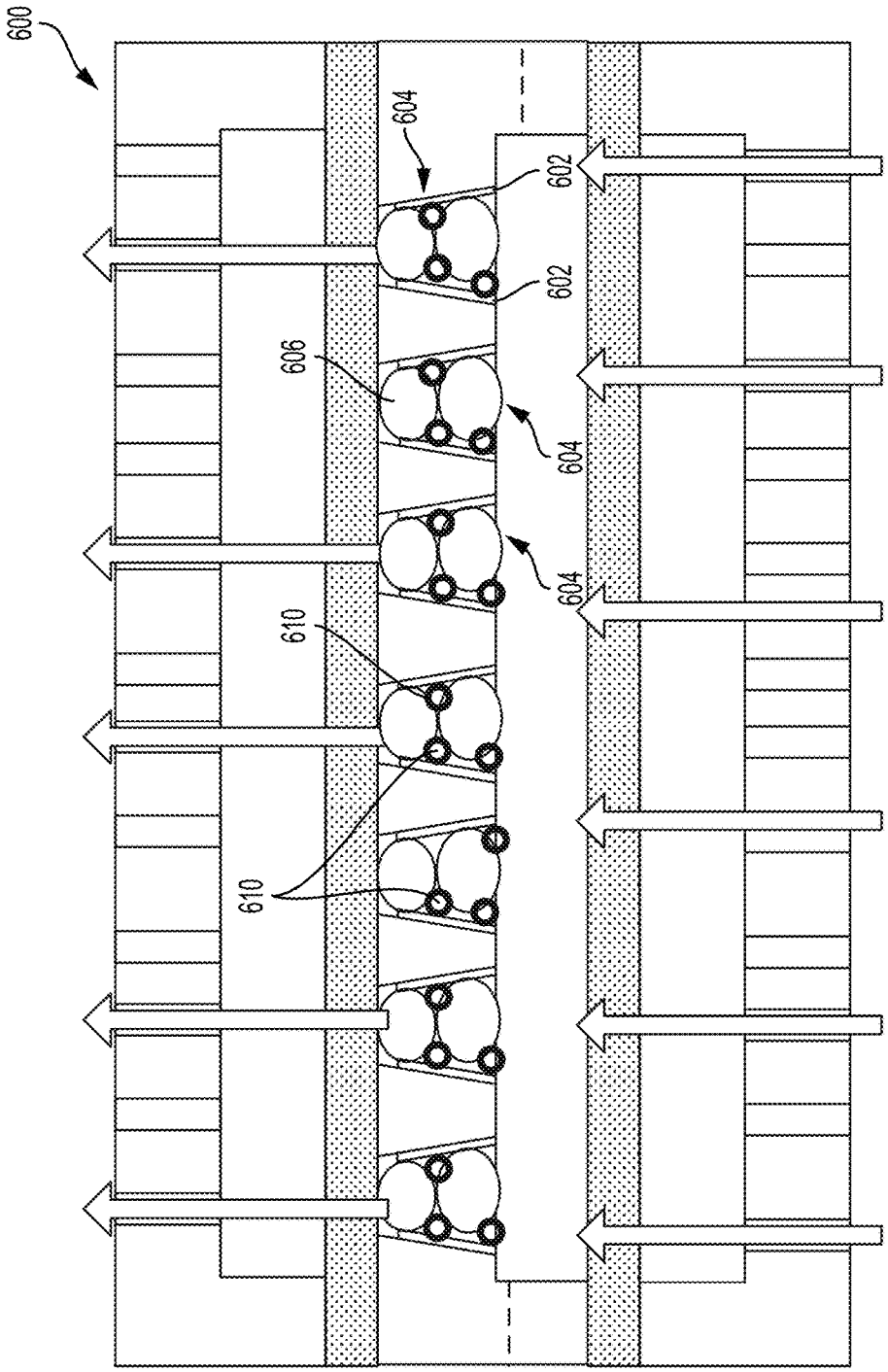
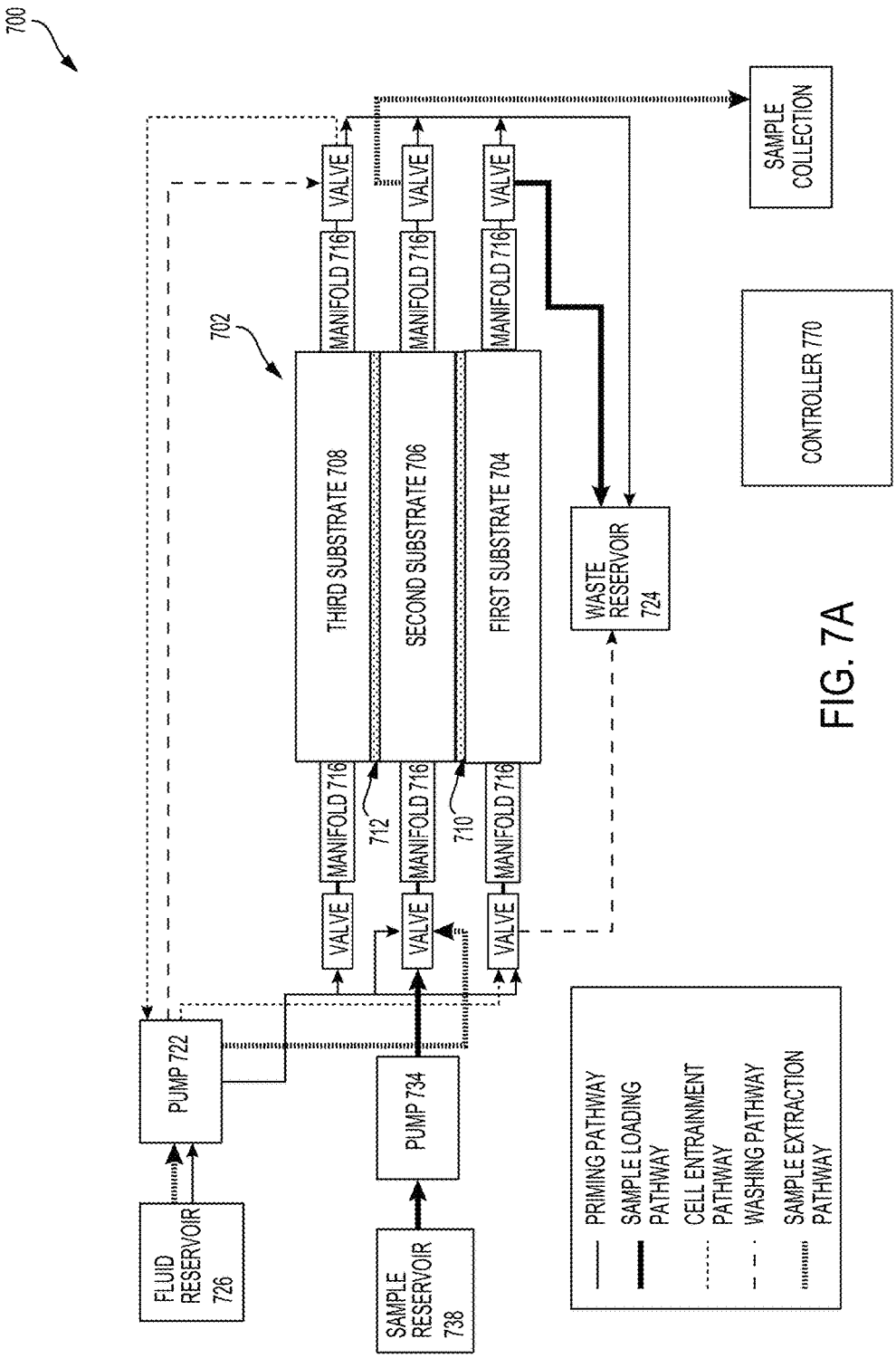


FIG. 6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CONTROLLER 770
VALVE
MANIFOLD 716
702
THIRD SUBSTRATE 708
SECOND SUBSTRATE 706
FIRST SUBSTRATE 704
WASTE RESERVOIR 724
712
710
PUMP 722
PUMP 734
FLUID RESERVOIR 726
SAMPLE RESERVOIR 738
PRIMING PATHWAY
SAMPLE LOADING PATHWAY
CELL ENTRAINMENT PATHWAY
WASHING PATHWAY
SAMPLE EXTRACTION PATHWAY

FIG. 7A

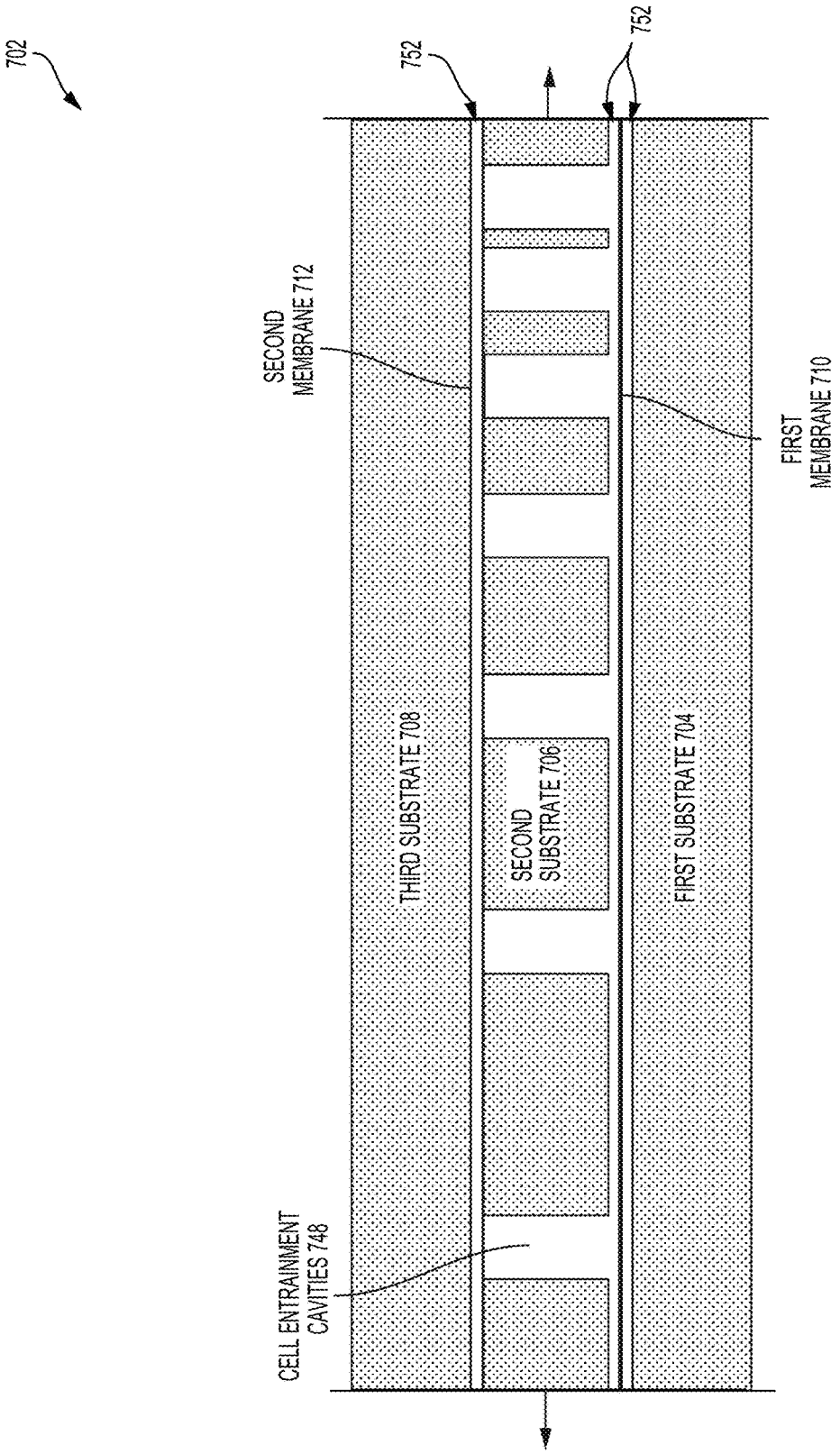
702
752
752
SECOND MEMBRANE 712
FIRST MEMBRANE 710
THIRD SUBSTRATE 708
SECOND SUBSTRATE 706
FIRST SUBSTRATE 704
CELL ENTRAINMENT CAVITIES 748

FIG. 7B

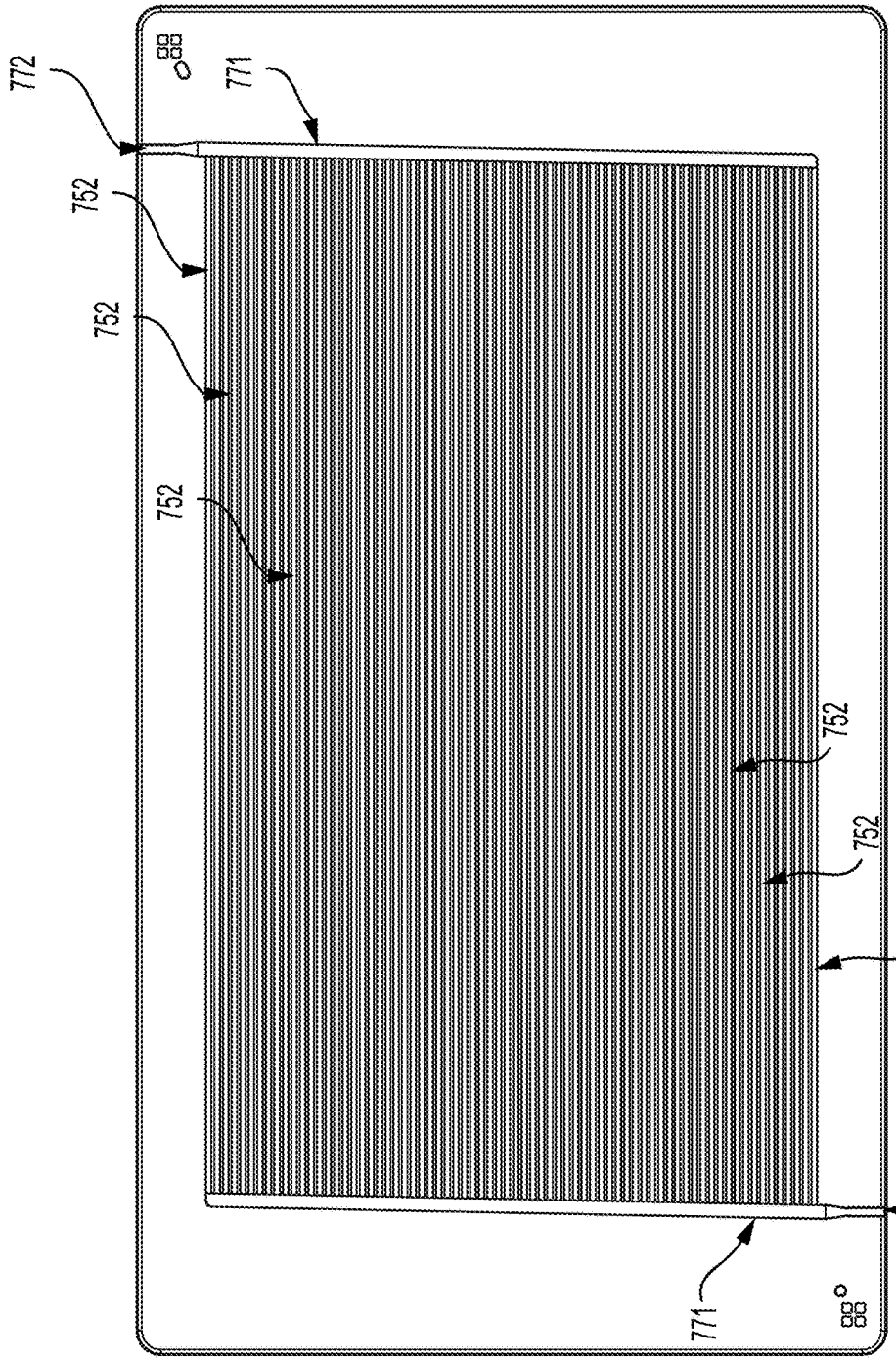

FIG. 7C

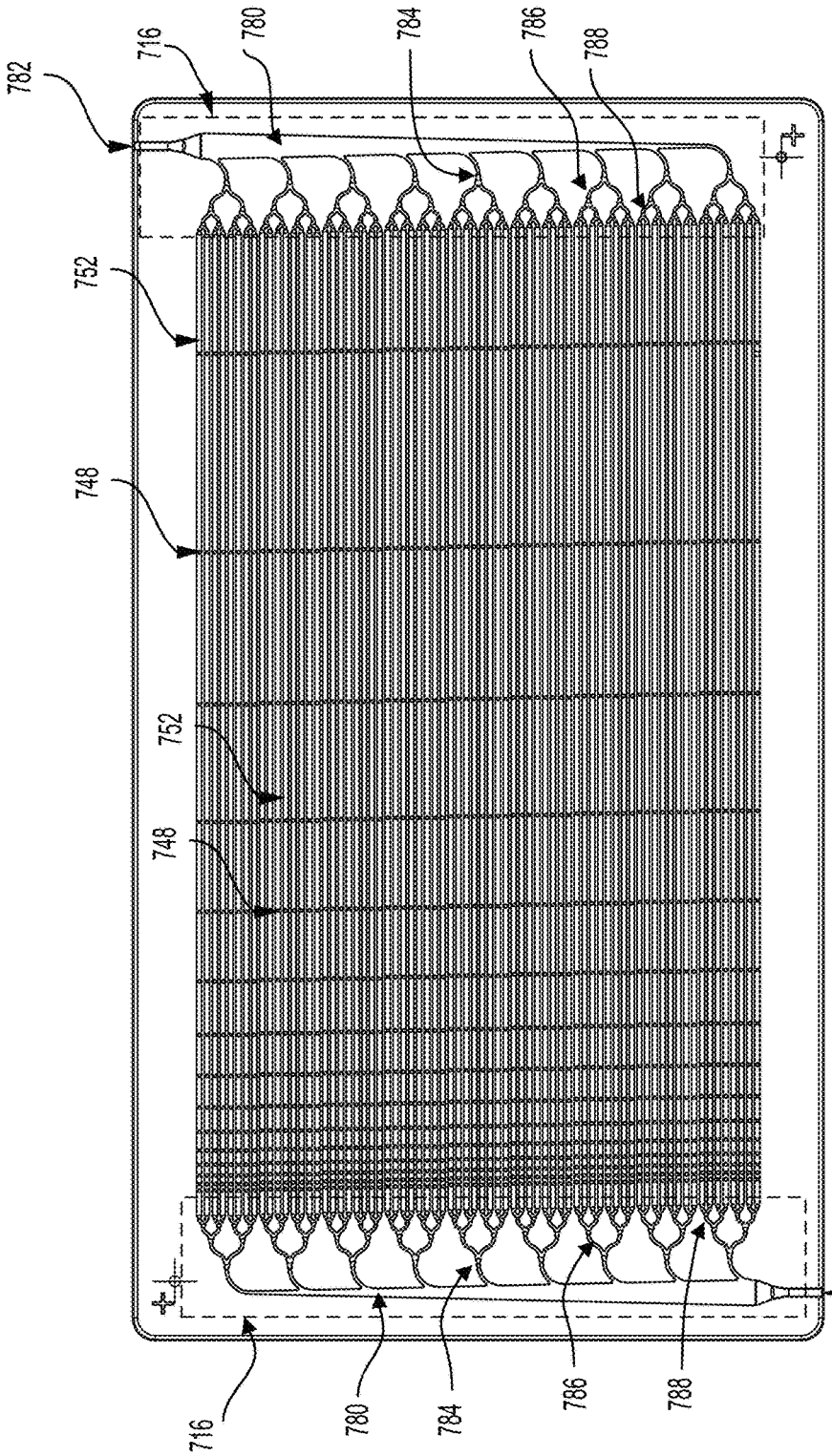

FIG. 7D

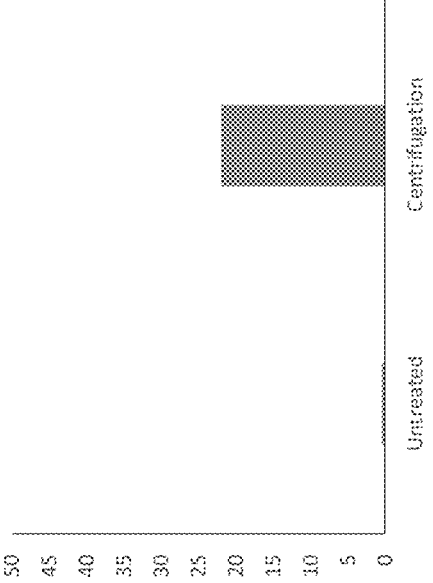

Figure X. Transduction efficiency of Jurkat cells after 30 minutes of exposure to a GFP + lentiviral vector (MOI 3, 1 million cells). Transduction efficiency was characterized by assessing total GFP+ cells/total cells.

FIG. 8

SYSTEMS AND METHODS FOR CELL TRANSDUCTION

RELATED APPLICATIONS

The present application claims the benefit of, and priority to, U.S. Provisional Patent Application No. 62/346,031, titled "Microfluidic Viral Transduction for Chimeric Antigen Receptor T Cell Technology and Other Cell Therapies," filed on Jun. 6, 2016, and U.S. Provisional Patent Application No. 62/421,784, titled "Systems and Methods for Cell Transduction," filed on Nov. 14, 2016, both of which are hereby incorporated by reference in their entirety.

BACKGROUND

Various treatments for a variety of medical conditions involve the transfer of exogenous genetic information into cells of a patient or a cell donor. For example, CAR-T (chimeric antigen receptor T cell) technology involves taking blood samples from a patient and processing those cells in a manner that returns genetically engineered populations of T cells to the patient's body once they have been programmed to recognize specific antigens on targeted cells. Typically, genes are transferred into T cells by viral transduction with a retrovirus (e.g., lentivirus), but they can also be transfected into cells using physical methods such as electroporation or cell constriction within channels, chemical methods, or other approaches.

SUMMARY

According to one aspect of the disclosure, an apparatus includes a first substrate defining at least one first flow chamber coupled to a first fluid manifold and a second substrate defining a cell entrainment layer. The cell entrainment layer includes at least one second flow chamber and a plurality of cell entrainment cavities. Each of the cell entrainment cavities opens at one end into one of the second flow chambers. Each of the cell entrainment cavities extends through the second substrate and is sized to hold at least one cell. The cell entrainment layer includes at least one inlet to the at least one second flow chamber that is substantially within the plane of the second substrate. The cell entrainment layer includes at least one outlet from the at least one second flow chamber that is substantially within the plane of the second substrate. The apparatus includes a first membrane positioned between the first substrate and second substrate. The first membrane includes a plurality of pores that are small enough to prevent the passage of cells and large enough to allow the passage of viral particles. The apparatus includes a third substrate defining at least one third flow chamber coupled to a second fluid manifold. The apparatus includes a second membrane positioned between the second substrate and the third substrate. The second membrane includes a second plurality of pores that are small enough to prevent passage of viral particles but large enough to allow the passage of cell media.

In some implementations, the at least one first flow chamber, the at least one second flow chamber and/or the at least one third flow chamber includes a respective substantially planar flow field that couples to a corresponding manifold via a plurality of fluid connections. In some implementations the at least one first flow chamber, the at least one second flow chamber and/or the at least one third flow chamber include a plurality of flow channels. Each flow channel couples to a corresponding manifold via a single fluid connection.

In some implementations, the at least one first fluid manifold and the second fluid manifold include a vertical flow manifold. In some implementations, the at least one first fluid manifold and the second fluid manifold include a horizontal flow manifold.

In some implementations, a first end of the first fluid manifold couples to the at least one first fluid chamber defined by the first substrate, and a first end of the second fluid manifold couples to the at least one third fluid chamber defined by the third substrate. A second end of the first fluid manifold is fluidically coupled to a second end of the second fluid manifold such that fluid can circulate through the first fluid manifold, the first membrane, the plurality of cell entrainment cavities, the second membrane, the second fluid manifold and back to the first fluid manifold. In some implementations, the apparatus includes a waste channel coupled between the second end of the first fluid manifold and the second fluid manifold by a valve. The valve is configured to selectively divert fluid flow directed out of the second end of the first fluid manifold to a waste reservoir.

In some implementations, the apparatus includes a first pump configured to pump fluid into the second end of the first fluid manifold. In some implementations, the apparatus includes a second pump configured to pump fluid into the second end of the second fluid manifold, and wherein the second pump is the same pump as the first pump or different than the first pump.

In some implementations, the first substrate includes an outlet coupled to a distal end of the at least one first fluid chamber.

In some implementations, the cell entrainment cavities have a greater density towards a distal end of the at least one second fluid chambers than towards a proximal end of the at least one second fluid chambers.

According to another aspect of the disclosure, a method of cell transduction includes introducing cells into at least one first flow chamber and introducing genetic information introduction agents into the first flow chamber. The method includes flowing a first fluid in a first direction substantially normal to the at least one first flow chamber and through a plurality of cell entrainment cavities distributed along the at least one first flow chamber having proximal ends open to respective first flow chambers, thereby entraining the introduced cells and genetic information introduction agents into the plurality of cell entrainment cavities for a first period of time, thereby allowing the genetic information carried by the genetic information introduction agents to be transduced into the entrained cells. The method includes preventing passage, through distal ends of the cell entrainment cavities, of the cells and the genetic information introduction agents. The method includes reversing the direction of flow of the first fluid for a second period of time, thereby releasing the cells from the cell entrainment cavities and washing the genetic information introduction agents away from the cells. The method includes flowing the released cells out of the at least one first flow chamber for collection.

In some implementations, the method includes flowing the first fluid through a first membrane having pores sized to prevent passage of the cells but large enough to allow passage of the genetic information introduction agents. In some implementations, the method includes flowing the first fluid through the distal end of the cell entrainment cavities through a second membrane having pores sized large enough to allow passage of first fluid and small enough to

prevent passage of the genetic information introduction agents through the second membrane. In some implementations, the method includes creating a circulating flow in which fluid flowing through the second membrane is redirected back through the first membrane in the first direction.

In some implementations, the method includes introducing the cells and the genetic information introduction agents into the first flow field substantially simultaneously. In some implementations, the method includes introducing the cells into the first flow field prior to the introduction of the genetic information introduction agents into the first flow field.

According to another aspect of the disclosure, an apparatus includes a first substrate defining at least one first flow chamber coupled to a first fluid manifold. The apparatus includes a second substrate defining at least one second flow chamber. The second flow chamber includes a first membrane positioned between the first substrate and the second substrate. The first membrane includes a plurality of pores that are small enough to prevent the passage of cells and large enough to allow the passage of a virus. The apparatus includes a third substrate defining a third flow chamber and coupled to a second fluid manifold. The apparatus includes a second membrane positioned between the second substrate and the third substrate. The second membrane includes a second plurality of pores that are small enough to prevent the passage of viral particles but large enough to allow the passage of cell media. The apparatus includes a means for entraining cells within the at least one second flow chamber as a result of a flow of fluid across the first and second membranes.

In some implementations, the means for entraining includes the second membrane. In some implementations, the second membrane includes one of a patterned membrane and an unpatterned membrane.

BRIEF DESCRIPTION OF THE DRAWINGS

The skilled artisan will understand that the figures, described herein, are for illustration purposes only. It is to be understood that in some instances various aspects of the described implementations may be shown exaggerated or enlarged to facilitate an understanding of the described implementations. In the drawings, like reference characters generally refer to like features, functionally similar and/or structurally similar elements throughout the various drawings. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the teachings. The drawings are not intended to limit the scope of the present teachings in any way. The system and method may be better understood from the following illustrative description with reference to the following drawings in which:

FIG. **1**A is a block diagram of an example cell transduction system;

FIG. **1**B shows a second example cell transduction system;

FIG. **1**C shows a top view of a transduction stack suitable for use in either of the cell transduction systems shown in FIGS. **1**A and **1**B;

FIG. **1**D shows another example manifold suitable for use as the first and fourth external fluid manifolds;

FIG. **1**E shows a perspective cut-away view of an example transduction stack suitable for use in the cell transduction systems shown in FIGS. **1**A and **1**B;

FIG. **2** shows a block diagram of an example method of transducing genetic information into cells;

FIGS. **3**A-**3**E show various stages of the execution of the method shown in FIG. **2** using a cell transduction stack suitable for use in the cell transduction systems shown in FIGS. **1**A and **1**B;

FIG. **4** shows a second example method of cell transduction using a cell transduction system similar to the cell transduction systems shown in FIGS. **1**A and **1**B;

FIGS. **5**A-**5**D show various stages of the method shown in FIG. **4** using a cell transduction stack suitable for use in the cell transduction systems shown in FIGS. **1**A and **1**B;

FIG. **6** shows a cross-sectional view of another example transduction stack;

FIGS. **7**A-**7**D show various views of another example implementation of a cell transduction system; and

FIG. **8** shows experimental results of executing the method shown in FIG. **2** using a cell transduction system similar to that shown in FIGS. **7**A-**7**D.

DESCRIPTION OF CERTAIN ILLUSTRATIVE IMPLEMENTATIONS

The various concepts introduced above and discussed in greater detail below may be implemented in any of numerous ways, as the described concepts are not limited to any particular manner of implementation. Examples of specific implementations and applications are provided primarily for illustrative purposes.

Systems and methods are disclosed herein for use in the transduction process involved in CAR-T (chimeric antigen receptor T-cell) and other cell modification or stimulation regimens. Other example uses of the technology disclosed include protein and virus production, cell expansion, reprogramming of stem cells, silencing of particular genes for treatment of genetic diseases, activation of T-cells, or siRNA delivery. Other uses of the systems and methods could be implemented without departing from the scope of this disclosure.

The devices discussed herein generally include three layers, separated from one another by membranes. Each layer defines at least one flow chamber. As used herein, a "flow chamber" refers to any conduit for carrying fluid across a layer in the device. Flow chambers can generally be classified as either flow channels or flow fields. As used herein, a "flow field" refers to a wider flow chamber which couples to a manifold via multiple fluid connections. In contrast, as used herein, a "flow channel" refers to a narrower flow chamber that couples to a manifold via a single fluid connection. Accordingly, in some implementations, each layer defines one or more flow fields. In some other implementations, each layer defines multiple flow channels. In some implementations, at least one layer includes one or more flow fields and one or more other layers includes multiple flow channels.

The layers of the devices discussed herein are configured to allow for a reversible vertical flow across the flow chambers defined in each layer in a direction substantially normal to the planes of the layers, themselves, as well as for horizontal flow at least through the central layer. The central layer defines cell entrainment regions in which cells and genetic information introduction agents or other chemical or biologic additives can be entrained to cause the genetic information carried by the genetic introduction agents (or the additives) to be introduced into (or otherwise interact with) the entrained cells. Such additional additives may include antibodies, cytokines, small molecules, proteins, or any other agent that might interact with the entrained cells. The horizontal flow through the central layer is used to

introduce cells and genetic information introduction agents or other additives into the central layer, distribute the cells and genetic information introduction agents or additives amongst the cell entrainment regions, and then remove cells from the central layer after treatment. The vertical flow across the layers is used to entrain the cells and genetic information introduction agents or additives into the cell entrainment regions. The vertical flow can then be reversed to release the cells from the cell entrainment regions and then wash away excess genetic introduction agents or additives.

In various embodiments, fluid flows are introduced into the flow chambers of the three layers of the device through either external fluid manifolds or integrated fluid manifolds. External fluid manifolds are formed in separate components and are fluidically coupled to the flow chambers via fluid passageways defined into the layers. Integrated fluid manifolds are formed directly into the material that makes up a particular layer. In some implementations, the fluid manifolds introducing fluid into each of the layers are horizontal fluid manifolds. In some implementations, the fluid manifolds introducing fluid into the outer two layers are vertical fluid manifolds.

In some implementations, fluids may be introduced into a given layer via an external manifold and removed through an integrated manifold, or vice versa. In some implementations, at one of both ends of a given layer, an external manifold may couple to an integrated manifold.

The two membranes in the device are selected to control the passage of fluid and biologic material between the layers of the device. The membranes can be generally impermeable, except through specifically defined pores through the membrane. The pores of one membrane are sized to be large enough to allow passage of fluid, such as cell media, but small enough to prevent passage of genetic information introduction agents or other additives introduced into the system. The pores of the other membrane are larger, allowing the passage of genetic information introduction agents or other additives introduced into the system, but are still small enough to prevent the passage of cells. In some implementations, the pores of both membranes may be large enough to allow genetic introduction agents or other additives to pass. In such implementations, genetic introduction agents or other additives recirculate through the device with the vertical flow.

FIG. **1**A is a block diagram of an example cell transduction system **100**. The system includes a transduction stack **102** and associated fluidics that control the flow of fluid into and out of the transduction stack **102**.

The transduction stack **102** includes a first substrate **104**, a second substrate **106**, and a third substrate **108**. The first substrate **104** is separated from the second substrate **106** by a first membrane **110**, and the second substrate **106** is separated from the third substrate **108** by a second membrane **112**.

In the example cell transduction system **100**, the first substrate **104** defines a first flow field which extends substantially in a plane that is parallel to the plane of the first substrate **104**. The first substrate **104** further defines a plurality of fluid passageways passing through a first side of the first substrate **104** opposite the first membrane **110**. The fluid passageways extend substantially normal to the planes of the first substrate **104** and the first flow field. The fluid passageways are distributed substantially evenly across the first side of the first substrate and fluidically couple the first flow field to a first external fluid manifold **114**. The first external fluid manifold **114** introduces fluid into the cell transduction stack **102** through a two dimensional array of fluid passages, allowing a fluid introduced by the cell transduction system **100** to be introduced in a substantially even manner across the first flow field in a direction normal to the planes of the first substrate **104** and the first flow field, yielding, in some implementations, a substantially uniform flow of fluid across the flow field.

The second substrate **106** defines a second flow field. The second flow field is likewise substantially planar and extends in a plane substantially parallel to the plane of the second substrate **106**. The second substrate **106** defines a plurality of inlets along a first edge of the second substrate, which fluidically couple the second flow field to outlets of a second external fluid manifold **116**. The second external fluid manifold distributes a second fluid along the edge of the second substrate such that second fluid enters the second flow field substantially evenly along a corresponding edge of the second flow field. The second fluid is introduced in a direction that is normal to the direction of the flow of the first fluid discussed above. That is, the second fluid is flowed within the plane of the second flow field. The second substrate further defines a plurality of outlets distributed along a second edge of the second substrate **106**, opposite the first edge. The outlets fluidically couple the second flow field to a third external fluid manifold **118**, which carries fluid out of the second flow field.

In some implementations, the second substrate **106** defines an array of cell entrainment cavities. The cell entrainment cavities can be formed from holes penetrating the second substrate **106** in a direction substantially normal to the plane of the second substrate **106**. The holes are sufficiently wide at the end proximate to the second flow field (the "proximate end") and deep enough to hold at least one cell. In some implementations, the holes are each sized and shaped to hold a single cell. In some implementations, the holes are sized and shaped to hold multiple cells ranging from one cell to thousands or even about a million cells. For example, the holes may be generally circular, hexagonal, octagonal, rectangular, elliptical, or have any other suitable shape. In some implementations, the proximate end may have a diameter of between about 0.01 mm to about 1.0 mm. In some implementations, the proximate end may have a diameter of between 0.1 mm and 1.0 mm. In some implementations, the proximate end may have a diameter of between about 0.50 and about 0.80 mm. The cell entrainment cavities can have depths ranging from about 0.01 mm to about 2.0 mm. In some implementations, the cell entrainment cavities are between about 0.1 mm and about 0.5 mm deep. In some implementations, the walls of the cell entrainment cavities are vertical (i.e., normal to the plane of the second substrate **106**). In some other implementations, the walls of the cell entrainment cavities are sloped, such that the cell entrainment cavities narrow as they approach their distal end, adjacent the second membrane **112**. The slope of the walls can range from about 45 degrees up to about 90 degrees. In some implementations, the walls can have a slope of between about 60 degrees to about 80 degrees. The cell entrainment cavities can be rather tightly packed across the second substrate **106**. In some implementations, the cavities can be arranged in a staggered fashion to maximize packing density. In some other implementations, the cavities can be arranged in a rectangular, hexagonal, or other geometric array. The space between cell entrainment cavities in any direction can be less than the diameter of the proximal end of the holes forming the cell entrainment cavities. In various implementations, the second substrate may define between about 1,000 cavities and about 10,000,000 cell

entrainment cavities. In some implementations, the cavities are regularly spaced along the length of the flow field. In some implementations, the cavities are irregularly spaced. For example, for implementations including flow channels, cavities can be more densely packed toward the distal end of the flow channels to ensure cells are likely to be entrained before reaching the end of the channel. For some implementations including flow fields, the density of cavities along the central axis of the flow field may be higher than towards the edges as cells are likely to migrate towards the center of the flow fields. In addition, or in the alternative, in some flow field implementations, the density of cavities may be greater at the distal end of the flow field than at the proximal end of the flow field. In some implementations, the cavities may be positioned such that a substantially equal number of cells are entrained in each cavities. The width of the cavities may be designed to house at least one cell, but may also be keyed to the width of the flow chamber to enable multiple cells in an individual cavity or to promote ease of manufacturing processes such as alignment.

In some other implementations, the second substrate **106** does not define cell entrainment cavities, and instead holds a porous gel or mesh adjacent to the second flow field, in which cells can become entrained. The porous gel or mesh may be impregnated with chemical factors, such as cytokines, and/or genetic information introduction agents, such as viruses, viral particles, plasmids, plasmid vectors, CRISPR complexes or any other means for introducing genetic information into a cell including agents of vector introduction such as lipofectamine. The gel or mesh is permeable to fluid flowing through the second flow field, and contains cavities within it which can entrain cells.

The third substrate **108** defines a third flow field and a second plurality of fluid passageways Like the first plurality of fluid passageways defined through the first substrate **104**, the second plurality of fluid passageways extend through third substrate **108** in a two-dimension array in a direction substantially normal to the plane of the third substrate **108**. The second plurality of fluid passageways fluidically couple the third flow field to a fourth external fluid manifold **120**.

While shown in FIG. **1**A as being coupled to external fluid manifolds, in some implementations, one or more, and in some cases, all of the substrates **104**, **106**, and **108** include integrated fluid manifolds. Example integrated manifolds are shown in FIGS. **7**C and **7**D.

Each of the first, second, and third substrates **104**, **106**, and **108** can be made of polystyrene, polycarbonate, polyimide, polyetherimide (PEI), polysulfone, polyethersulfone, acrylic, or cyclic olefin copolymer (COC), biodegradable polyesters, such as polycaprolactone (PCL), soft elastomers such as polyglycerol sebacate (PGS), other thermoplastics or other structural materials. The substrates may alternatively be made of polydimethylsiloxane (PDMS), poly(N-isopropylacrylamide), polyurethane (PU), fluorinated ethylene propylene (FEP), or a fluoropolymer elastomer. In some implementations, one or more of the first, second, and third substrates **104**, **106**, and **108** can be formed from glass, a ceramic, or a semiconductor, such as Silicon (Si). The substrates **104**, **106**, and **108** can range from about 0.5 mm to about 4 mm thick. In some implementations, the substrates are between about 0.5 and about 2.0 mm thick. The combined set of flow chamber(s) for a given layer, including one or more parallel flow fields or flow channels, can be generally rectangular or square shaped with dimensions running from about 5 mm wide by about 5 mm long by about 0.1 mm deep to about 20 cm long by about 20 cm wide by about 2 mm deep. In some implementations the length:width ratio of the combined set of flow chambers in a layer is about 1:1. In some implementations, one or more of the flow chambers have a circular, oval, hexagonal, or other geometric or irregular shape. In some implementations, instead of including one or wider flow fields in each layer of the transduction stack **102**, or ore more of the layers can include a greater number of parallel flow channels. In some implementations, multiple cell transduction stacks **102** can be connected to the fluidics in parallel to allow for the processing of more cells at a time.

The first membrane **110** separates the first flow field defined by the first substrate **104** from second flow field defined by the second substrate **106**. The membrane can be formed from a generally fluid impermeable material, such as polycarbonate, PET, or various dialysis membranes. In some implementations, the membrane material is either hydrophilic, or one or both sides of the first membrane **110** is coated with a hydrophilic material such as PVP (polyvinylpyrrolidone). Pores are formed, for example by track etching, through the first membrane **110** that are sized to be sufficiently large to allow genetic information introduction agents, such as viruses, virus particles, plasmids, CRISPR complexes, or other nucleic acid delivery agents to pass through the first membrane **110**, i.e., at least about 0.1 microns and less than about 1.0 micron in diameter. In some implementations the pores are about 0.4 microns in diameter. Pores may also be formed by other techniques such as micromolding from a master mold, or by precipitation, sacrificial methods, or other techniques that produce tortuous path pores in the membranes. The first membrane **110** can have a pore density of about 15 to about 30 percent.

The second membrane **112** is similar to the first membrane **110**, and separates the second substrate **106** from the third flow field. The pores of the second membrane **112**, however, are smaller in diameter than the pores in the first membrane **110**. The pores in the second membrane **112**, for example, can be smaller than the smallest genetic information introduction agent intended to be used in the system **100**. For example, the pores in the second membrane can be between about 0.001 micron and about 0.5 micron in diameter. In some implementations, the pores in the second membrane **112** are about 0.1 microns in diameter. The second membrane **112** can have a pore density of about 15 to about 30 percent. The first and second membranes **110** and **112** can be between about 8 microns and about 12 microns thick, for example about 10 microns thick.

The fluidics in the cell transduction system **100** include a vertical flow system configured to flow fluid through the transduction stack **102** bi-directionally, substantially normal to the first, second, and third substrates **104**, **106**, and **108**. The vertical flow system includes a three-port pump **122**, a three-port valve **124**, the first and fourth external fluid manifolds **114** and **120**, a fluid reservoir **126**, a waste reservoir **128**, and connecting fluid channels. The three-port pump can draw fluid, such as cell media from the fluid reservoir **126** and pump it through the transduction stack **102**. The three port pump pumps the fluid through the transduction stack **102** such that the fluid enters the transduction stack **102** either through the first external fluid manifold **114** and the first substrate **104** or through the fourth external fluid manifold **120** and the third substrate **108**. In one mode of operation, in which the three-port valve isolates the waste reservoir **128** from the remainder of the vertical flow system, and once a sufficient amount of fluid has been introduced into the vertical flow system from the fluid reservoir **126**, the three-port pump **122** can isolate the fluid reservoir **126** from the remainder of the vertical flow system,

and can recirculate the fluid through the transduction stack **102** in the direction shown by arrow **130** (i.e. counterclockwise in the figure). In another mode of operation, in which the three port valve fluidically couples the first external fluid manifold **114** to the waste reservoir and closes the fluid path between the first external fluid manifold **114** and the three-port pump **122**, the three-port pump **122** opens the fluid path to the fluid reservoir **126** and reverses the direction of flow through the transduction stack **102**, as shown by the arrow **132**. In this mode of operation, fluid from the fluid reservoir **126** flows into the transduction stack **102** from the fourth external fluid manifold **120**, out through the first external fluid manifold **114**, into the waste reservoir **128** through the three-port valve.

The fluidics of the cell transduction system **100** also includes a horizontal flow system. The horizontal flow system is configured to introduce cells (and in some implementations genetic information introduction agents) into the second flow field defined in the second substrate **106** of the transduction stack **102**. The horizontal flow system introduces the cells in a direction that is within the plane of the second flow field. The horizontal flow system includes a pump **134**, an outlet valve **136**, a sample reservoir **138**, the second and third external fluid manifolds **116** and **118**, and connecting fluid channels.

In some implementations, the pump **134** is a three-port pump. In such implementations, a first port couples to the sample reservoir **138**, a second port couples to the fluid reservoir **126**, and a third port couples to the second external fluid manifold. The pump **134**, in such implementations can either pump fluid from the sample reservoir, including, for example cells and genetic information introduction agents suspended in cell media, or fluid form the fluid reservoir **126** into the transduction stack **102** through the second external fluid manifold **116**.

In implementations in which the pump **134** is a four-port-pump, the fourth port of the pump couples to the outlet valve **136**. In such implementations, fluid can be recirculated through the second flow field, out through the third external fluid manifold **118**, through the outlet valve **136**, and back to the pump **134**. Such implementations can be useful if an insufficient number of cells or number of genetic information introduction agents are successfully entrained in cell entrainment cavities adjacent the second flow field as the fluid from the sample reservoir **138** makes a first pass through the second flow field. Cells or genetic information introduction agents that are not entrained can be recirculated through the second flow field in a recirculating flow to allow more of the cells and genetic information introduction agents to become entrained.

The outlet valve **136** is configured so that it also can be closed, completely preventing any flow through the outlet valve, or opened to a system output from which transduced cells can be collected.

In some other implementations, instead of being entrained in a substrate cavity, gel, or mesh, the cells can be entrained directly up against the second membrane **112**. In some implementations, the second membrane **112** may be patterned to form a relief with raised regions and lower regions, to enhance the ability of the membrane to entrain cells. The lower regions can have dimensions on the order of 0.01 microns to 0.8 microns. One or more of the cells can be entrained within these lower regions, depending on their relative sizes. In some implementations, an unpatterned second membrane can serve as a means for entraining cells.

FIG. **1**B shows a second example cell transduction system **150**. The cell transduction system **150** is substantially similar to the cell transduction system **100** with the following differences. First, in addition to the first plurality of fluid passageways, the first substrate **104'** of the cell transduction system **150** includes one or more outlets along one edge of the first flow field, allowing an alternate path for fluid to escape the first flow field. The waste reservoir **128** of the cell transduction system **150** is coupled to the one or more outlets instead to the valve **124**. In some implementations in which the first substrate **104'** defines multiple outlets along its edge, the cell transduction system **150** may include a fifth external fluid manifold between the first substrate **104'** and the waste reservoir to combine the outflows from the first substrate **104'**. The cell transduction system **150** can include a second valve **152** between the first substrate **104'** and the waste reservoir **128** to gate the flow of fluid therebetween. Given the different location of the waste reservoir **128**, instead of including a three-port valve between the pump **134** and the first external fluid manifold, the cell transduction system **150** uses a two-port valve **124'**, which either allows flow through the valve, or prevents its flow.

Each of the cell transduction systems **100** and **150** can also include a controller **170** configured to control the pumps and valves included therein to carry out the functionality and methods described herein. For example, the controller **170** can be a special purpose or general purpose processor executing computer executable instructions configured to carry out the herein disclosed methods, either automatically, or in response to user interactions.

The differences in operation between the cell transduction system **100** shown in FIG. **1**A and the cell transduction system **150** shown in FIG. **1**B is described further below in relation to FIGS. **3**C and **3**D.

FIG. **1**C shows a top view of a transduction stack **102** suitable for use in either of the cell transduction systems **100** and **150** shown in FIGS. **1**A and **1**B. Specifically, FIG. **1**C shows examples of the second and third external fluid manifolds **116**, **118** coupled to an example second substrate (not shown) and an example fourth external fluid manifold **120** coupled to an example of the third substrate **108**. The example fourth external fluid manifold **120** shown in FIG. **1**C distributes fluid across the top of the third substrate **108** through channels of a fourth substrate that couple to through-holes that match up to the second plurality of openings in the third substrate **108**. In some implementations a similar fluid manifold can be used for the first external fluid manifold.

FIG. **1**D shows another example manifold suitable for use as the first and fourth external fluid manifolds **114** and **120**. The fluid manifold in FIG. **1**D is three dimensional in nature and distributes fluid in three-dimensions (though its outputs are still arranged in two dimensions), whereas the example fourth external fluid manifold shown in FIG. **1**C distributes fluid primarily only in two dimensions.

FIG. **1**E shows a perspective cut-away view of an example transduction stack **102** suitable for use in the cell transduction systems **100** and **150**. Like reference numerals refer to like features in FIGS. **1**A and **1**B. FIG. **1**E shows examples of the first flow field **142**, second flow field **144**, third flow field **146**, and cell entrainment cavities **148**, not shown in FIGS. **1**A and **1**B.

FIG. **2** shows a block diagram of an example method **200** of transducing genetic information into cells. The method **200** can be implemented, for example, using the cell transduction systems **100** and **150** shown in FIGS. **1**A and **1**B. The method **200** includes introducing cells into a first flow field (step **202**), introducing genetic information introduction agents into the first flow field (step **204**), and entraining

the introduced cells and the introduced genetic information introduction agents into cell entrainment cavities (step **206**), while preventing passage of the cells and the genetic information introduction agents through a distal end of the cell entrainment cavities (step **208**). The method **200** further includes releasing the cells from the entrainment cavities (step **210**), washing the genetic information introduction agents from the released cells (step **212**), and collecting the released, washed cells (step **214**). Each of the above steps will be described further below with reference to FIGS. **1**A and **1**B and FIGS. **3**A-**3**E, which illustrate the various steps of the method **200**.

The method **200** includes introducing cells into a first flow field (step **202**). The first flow field referenced in the method **200** can be, for example, the second flow field **144** defined by the second substrate **106** of the cell transduction systems **100** and **150**. The cells, in some implementations, can be T cells selected for transduction as part of a CAR-T cell immunotherapy regimen. Other suitable cell types include epithelial cells, endothelial cells, cancer cells, hematopoietic stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonic stem cells for use in gene editing, ex-vivo gene therapy, and stem cell reprogramming applications. The cells can be introduced while suspended in a fluid, such as cell media. The media containing the cells can be pumped through the horizontal flow system discussed above by the pump **134**. The cells can be pumped from the sample reservoir **138** through the second external fluid manifold **116**, and into the second flow field **144**. In some implementations, the valve **136** is set to direct fluid that exits the second flow field back to the pump **134** to create a recirculating flow so that a sufficient number of cells can be entrained within the second substrate **106** (as discussed further below in relation to step **206**). In some implementations, cell media, absent any cells, is first pumped from the fluid reservoir **126** through the horizontal flow system before the cells are introduced to prime the transduction stack **102**.

Genetic information introduction agents, such as viruses, viral vectors, lipid nanoparticles, plasmids, CRISPR complexes, or other nucleic acid vectors are also introduced into the flow field (step **204**). In some implementations the genetic information introduction agents are suspended in the same fluid as the cells in the sample reservoir **138**. In some implementations, the sample reservoir **138** include separate compartments, keeping cells and genetic information introduction agents separated from one another until pumped into the transduction stack, and flows from the compartments combine as they flow through the horizontal flow systems of the cell transduction systems **100** or **150**. In some implementations the quantity of genetic information introduction agents in the fluid entering the second flow field **144** is sufficient to produce a vector copy number of about 1 per cell. In some implementations, the quantity of genetic information introduction agents in the fluid entering the second flow field **144** is sufficient to obtain an average vector copy number across the cell population of about 0.5 to about 2.5.

In some implementations, the cells and the genetic information introduction agents are introduced (steps **202** and **204**) into the flow field simultaneously. In some other implementations, the introduction of cells (step **202**) and genetic information introduction agents (step **204**) are carried out serially. In some implementations, the cells are introduced into the flow field before the genetic information introduction agents. In some implementations, the genetic information introduction agents are introduced into the flow field before the cells.

The method further includes entraining the introduced cells and genetic information introduction agents into cell entrainment cavities (step **206**). For example the introduced cells and genetic information introduction agents can be entrained into the cell entrainment cavities **148** shown in FIG. **1**E. The introduced cells and genetic information introduction agents are entrained as a result of fluid flow driven by the vertical flow system of the cell transduction systems **100** or **150**. That is, the pump **122** pumps fluid, such as cell media, through the first external fluid manifold **114**, vertically through the transduction stack **102** and out through the fourth external manifold **120**. In some implementations, the fluid is flowed at a rate of about 0.05 ml/minute to about 0.2 ml/minute. The flow results in a pressure gradient across the second membrane **120** of about 2 mm Hg to about 1000 mm Hg. In general, the pressure is maintained to be below about 750 mm Hg. In some implementations, the vertical flow is caused while the cells and the introduced cells, genetic information introduction agents, and/or additives are being flowed into the second flow field **144**. In some other implementations, the vertical flow is caused after the cells and the introduced cells, genetic information introduction agents, and/or additives have already been introduced into the second flow field **144**.

FIG. **3**A shows an example of the simultaneous introduction of cells **302** and genetic information introduction agents **304** into the second flow field **144** of a cell transduction stack **102** (steps **202** and **204**) via a horizontal fluid flow **306**. FIG. **3**A also shows the entrainment of the cells **302** and genetic information introduction agents **304** in cell entrainment cavities **148** via vertical flow **308***a* (step **206**). While FIG. **3**A shows cell entrainment cavities **148** each holding two cells, in various implementations, the cell entrainment cavities can be sized to hold between a single cell and thousands or even about a million cells.

As shown in FIG. **3**B, the cells **302** and genetic information introduction agents **304** remain entrained in the cell entrainment cavities for a dwell time sufficient to allow the genetic information carried by the genetic information introduction agents **304** to be introduced into the cells **302**, but not so long as to endanger the viability of the cells **302**. Accordingly, in some implementations, the dwell time is between about 5 minutes to about 7 hours. In some implementations, the dwell time set to be between about 10 minutes and about 2 hours. In some implementations, the dwell time is set to about 30 minutes. During this time, the vertical flow **308***a* through the transduction stack **102** is maintained, while preventing the passage of the cells **302** and genetic information introduction agents **304** through the distal ends **310** of the cell entrainment cavities (step **208**). The passage is prevented by the second membrane **112**, which has pores sufficiently large to allow the fluid of the vertical flow to pass through without building up too much pressure, but which are sufficiently small, for example between about 0.001 and about 0.5 microns in diameter, to prevent passage of the cells **302** and the smallest introduced genetic information introduction agents. During the dwell time, the horizontal flow **306** is halted and the valve **136** is closed.

Referring back to FIG. **2** and FIG. **3**C and **3**D, after the aforementioned dwell time, the cells **304** are released from the cell entrainment cavities **148** (step **210**) and the genetic information introduction agents are washed from the cells (step **212**). FIG. **3**C shows an example implementation of these steps in a cell transduction stack **102** of the form shown in the cell transduction system **100** shown in FIG. **1**A. FIG. **3**D shows a second example implementation of these

steps in a cell transduction stack **102'** of the form shown in the cell transduction system **150** shown in FIG. **1**B. As can be seen in FIGS. **3**C and **3**D, after the dwell time, many if not all of the cells become genetically modified or activated.

In both examples, the cells **304** are released (step **210**) and washed (step **212**) by the pump **122** reversing the direction of the vertical flow **308***a* to form a reverse vertical flow **308***b*. As such, the fluid in the vertical flow system enters the cell transduction stack **102** or **102'** via the fourth external fluid manifold **120** instead of the first external fluid manifold **114**. The fluid introduced in this reverse flow is drawn from the fluid reservoir **126**, and is not recirculated through the vertical flow system, thereby preventing the reintroduction of the genetic information introduction agents into the transduction stack. In the example shown in FIG. **3**C, recirculation is prevented by switching the three port valve **124** to redirect flow leaving the cell introduction stack into the waste reservoir **128**. In the example shown in FIG. **3**D, recirculation is prevented by closure of the valve **124'** and opening of the second valve **156**, allowing an alternate path for the fluid to flow out of the transduction stack **102** to the waste reservoir **128**.

As discussed above in relation to FIG. **1**A, the first membrane **110** includes pores that are large enough to allow the genetic information introduction agents **304**, but not the cells **302** from passing through. Thus the reverse vertical flow **308***b* washes the genetic information introduction agents **304** from the cells **302** and out of the transduction stack, either through the first external fluid manifold **114** (shown in FIG. **3**C) or though outlets **314** defined through an edge of the first substrate **104** (shown in FIG. **3**D). The reverse vertical flow **308***b* can have a similar flow rate as the vertical flow **308***a*, e.g., between about 0.05 and 0.2 ml/minute. The cells **302** can be washed for between about 30 seconds and about 15 minutes.

After the wash step (step **212**), the cells are collected (step **214**). The cells **302** are collected by the pump **134** reinitiating fluid flow through the horizontal flow system discussed in relation to FIG. **1**A, forcing the cells **302** out of the transduction stack **102**. In some implementations, the reverse vertical flow **308***b* is maintained while the cells are collected to prevent cells from getting caught in the cell entrainment cavities **148** as they exit the device. In some other implementations, the reverse fluid flow **308***b* is halted while the cells are collected. FIG. **3**E shows an example collection of transduced cells (step **214**).

In some implementations, prior to collection, the method **200** is repeated, with cells being recirculated back into the transduction stack and being introduced to another set or sets of genetic information introduction agents. In some such implementations, the additional set(s) of genetic information introduction agents carry the same additional genetic information as prior sets of genetic information introduction agents introduced into the transduction stack. In some other implementations, at least one additional set of genetic information introduction agents includes different genetic information to be introduced into the cells that prior genetic information introduction agents, thereby allowing for serial incremental introduction of genetic information into the cells.

FIG. **4** shows a second example method **400** of cell transduction using a cell transduction system similar to the cell transduction systems **100** and **150** shown in FIGS. **1**A and **1**B. In the method **400**, instead of entraining cells and genetic information introduction agents into cell entrainment cavities defined in a substrate of a transduction stack, cells are flowed into the transduction stack and then are entrained in a porous mesh or gel which had been previously impregnated with genetic information introduction agents, e.g., during manufacture of the device. In implementations in which the genetic information introduction agents are introduced into the gel or mesh prior to use of the device, the transduction stack can be maintained in a suitable environment (such as a refrigeration unit, incubator, or other environment control device) to maintain the viability of the genetic information introduction agents until use. Accordingly, the method **400** includes introducing cells into a first flow field adjacent to a mesh or gel impregnated with genetic information introduction agents (step **402**), entraining the introduced cells into the mesh or gel (step **404**), preventing passage of cells through the opposite side of the gel or mesh (step **406**), releasing the cells from the gel or mesh (step **408**), and collecting the released cells (step **410**). An example of this process is shown in FIGS. **5**A-**5**D. In other implementations, the genetic information introduction agents are flowed into the transduction stack via the horizontal flow system before, after, or concurrently with the introduction of cells such that the genetic information introduction agents are entrained within the gel or mesh in intimate contact with the cells.

FIG. **5**A shows the introduction of cells **502** into a flow field **504** adjacent a gel or mesh **506** impregnated with genetic information introduction agents **508** (step **402**) and the entrainment of the cells **502** into the gel or mesh **506** (step **404**). The cells **502** are introduced into the flow field (step **402**) via a horizontal flow **510** generated by the pump **134** of the horizontal flow system of the cell transduction system **100** or **150**, and are entrained by a vertical flow **512** generated by the pump **122** of the vertical flow system of the cell transduction system **100** or **150**. The flow rates can be similar to those discussed above for the vertical flow and reverse vertical flow **308***a* and **308***b*.

As shown in FIG. **5**B, after a sufficient number of cells **502** are entrained in the mesh or gel **506**, the horizontal flow **510** is halted while the vertical flow **512** is maintained for a dwell time similar in length to the dwell times discussed above with respect to FIGS. **2** and **3**B, to allow time for the genetic information introduction agents to introduce their genetic information into the cells **502**. The second membrane **112** prevents the cells and any genetic information introduction agents that are dislodged from the gel or mesh **506** from passing through an opposite side of the gel or mesh **506** (step **406**).

After the dwell time, the direction of the vertical flow **512** is reversed to form a reverse vertical flow **512***b*. The reverse vertical flow **512***b* releases the cells from the gel or mesh **506** (step **408**) and washes away any genetic information introduction agents that may have been dislodged from the gel or mesh **506**. The release step (step **408**) is shown in FIG. **5**C. As can be seen in FIG. **5**C, many if not all of the cells **502** now hold additional genetic information **514**. As shown in FIG. **5**D, the method **400** further includes collecting the released cells (step **410**), by reinitiating horizontal flow through the flow field **504**.

FIG. **6** shows a cross-sectional view of another example transduction stack **600**. The transduction stack **600** is similar to the transduction stack **102** shown in FIGS. **3**A-**3**E. However, the transduction stack **600** includes the additional features of electroporation electrodes **602** disposed on the walls of its cell entrainment cavities **604**. If a sufficient voltage is applied across the electrodes **602** while cells **606** are entrained in the cavities **604**, the cell membranes of the cells **604** will temporarily become more permeable, allowing a more passive introduction of genetic material, such as

plasmids **610**, CRISPR complexes, lipid nanoparticles or other nucleic acid or synthetic nucleic acid vectors.

FIGS. **7**A-**7**D show various views of another example implementation of a cell transduction system **700**. FIG. **7**A is a block diagram of the example cell transduction system **700**. FIG. **7**B is a cross-sectional view of an example cell transduction stack **702** suitable for inclusion in the cell transduction system **700**. FIG. **7**C shows a plan view of an example substrate suitable for use as the first or third substrates **704** and **708** in the cell transduction stack **702**. FIG. **7**D shows a plan view of an example second substrate **706** suitable for use in the transduction stack **702**. The cell transduction system can be operated, in some implementations, according to the method **200**, shown in FIG. **2**.

Referring to FIG. **7**A-**7**D and FIGS. **1**A-**1**E, the cell transduction system **700** is similar to the cell transduction system **100** Like the cell transduction system **100**, the cell transduction system includes a cell transduction stack **702** formed from three substrates, a first substrate **704**, a second substrate **706**, and a third substrate **708**. The first substrate **704** is separated from the second substrate **706** by a first membrane **710**. The second substrate **706** is separated from the third substrate **708** by a second membrane **712**. The first and second membranes **710** and **712** of the cell transduction system **700** can be substantially similar to the first and second membranes **110** and **112** used in the cell transduction system **100**. That is the membranes **710** and **712** can be made of the same materials described as suitable for the membranes **110** and **112**, the pore sizes for the first membrane **710** can be same as those described above for the first membrane **110**, and the sizes of the pores in the second membrane **712** can be the same as those described above for the second membrane **112**.

Like the cell transduction system **100**, the cell transduction system **700** also includes a fluid reservoir **726**, a sample reservoir **738**, and a waste reservoir **724**, which can have similar configurations to the fluid reservoir **126**, the sample reservoir **138**, and the waste reservoir **124** described above. The cell transduction system **700** also includes two pumps **722** and **734** and various valves for pumping and directing fluid, cells, and genetic information introduction agents (or other additives) through the cell transduction stack **702**. The pumps **722** and **734** can be controlled by a controller **770**.

In contrast to the cell transduction system **100**, the cell transduction system **700** lacks any vertical fluid manifolds, such as the manifolds **114** and **120**. Instead, all three substrates **704**, **706**, and **708** include integrated horizontal fluid manifolds **716** at both ends. As described above, an integrated fluid manifold refers to a fluid manifold formed in the same substrate as a set of flow chambers of the device, instead of being formed in a separate distinct substrate or other component. In addition, as can be seen best in FIGS. **7**C and **7**D, the flow chambers in the first, second, and third substrates **704**, **706**, and **708** are formed from multiple parallel fluid channels **752**, instead of single, wider flow fields **142**, **144**, and **146** shown in FIG. **1**E.

The various routes that fluid, cells, and genetic information introduction agents (or other additives) are directed through the cell transduction system **700** are also shown in FIG. **7**A. These include a priming pathway through which the system **700** is primed with buffer and then with cell media prior to introduction of cells or genetic information introduction agents (and/or other additives) and a sample loading pathway via which cells and genetic information introduction agents (and/or other additives) are loaded into the transduction stack **702**. FIG. **7**A also shows a cell entrainment pathway via which fluid, such as cell media, is flowed vertically (i.e., normal to the planes of the first, second, and third substrates **704**, **706**, and **708**) through the transduction stack **702** to entrain the cells and the genetic information introduction agents (or other additives) in cell entrainment cavities **748** (shown, for example, in FIGS. **7**B and **7**D). FIG. **7**A also shows a washing pathway via which a fluid, such as cell media, is flowed along a reversed vertical flow through the transduction stack **702** to release the cells from the cell entrainment cavities **748** and to wash the genetic information introduction agents (or other additives) from the released cells, through the first membrane **710**, and into the waste reservoir **724**. Finally, a cell extraction pathway is shown via which the washed cells can be removed from the cell transduction stack **702** and collected in a sample collection reservoir **740**.

FIG. **7**B shows a cross section of an example cell transduction stack **702** suitable for use in the cell transduction system **700**. The cross section is taken along the length of the cell transduction stack **702** (i.e., from left to right across the transduction stack **702** shown in FIG. **7**A). The cross-sectional view cuts through a flow channel formed in each the first, second, and third substrates **704**, **706**, and **708** and several cell entrainment cavities **748**. Each of the substrates **704**, **706**, and **708** can range from about 0.5 mm to about 4 mm thick. In some implementations, the substrates **704**, **706**, and **708** are between about 0.5 and about 2.0 mm thick. The substrates **704**, **706**, and **708** can have lengths and widths than range from about 5.0 cm to about 25 cm. In some implementations, the substrates **704**, **706**, and **708** have roughly equal lengths and widths. In some implementations, the substrates **704**, **706**, and **708** can be between 50%-200% longer (i.e., parallel to the axes of the fluid channels **752**) than they are wide (i.e., normal to the axes of the fluid channels **752**). In some implementations, the substrates **704**, **706**, and **708** are wider than they are long.

The fluid channels **752** formed in the first substrate **704** can be between about 100 microns and about 200 microns (for example, about 140 microns) deep, between about 50 microns and 1.0 mm (for example, about 800 microns) wide, and between about 10 cm to about 20 cm (for example, about 15 cm) long. The fluid channels **752** formed in the second substrate **706** can have similar lengths and widths to the fluid channels **752** formed in the first substrate **704**, but, in some implementations, are shallower. For example, the fluid channels **752** formed in the second substrate **706** can have depths between about 50 microns and 150 microns (e.g., between about 60 microns and about 70 microns). The fluid channels **752** in the third substrate **708**, in some implementations, have the same dimensions as the fluid channels **752** in the first substrate **704**.

As shown in FIG. **7**B, the fluid entrainment cavities **748** can be distributed unevenly along the length of the second substrate **706**. For example, the distance between adjacent cell entrainment cavities **748** along the length of the fluid channels **752** of the second substrate **706** can decrease towards the distal (with respect to the cell entrainment pathway shown in FIG. **7**A) end of the transduction stack **702**. The cell entrainment cavities can be circular, square, or any other regular or irregular shape sized to fit between one and about a million cells. For example, in the implementation shown in FIG. **7**B, cell entrainment cavities can have a diameter or width that is less than or about equal to the width of the channels **752**, in the range, for example, between about 500 microns to 1.0 mm (e.g., about 660 microns). The cell entrainment cavities **748** open on one onto the fluid channels **752** of the second substrate **706** and pass through the remaining of thickness of the second substrate **706**.

FIG. 7C shows a plan view of an example substrate suitable for use as the first and third substrates **704** and **708** of the cell transduction stack **702** shown in FIG. **7**A. As shown in FIG. **7**C, the example substrate includes integrated manifolds **716** at either end, connected by fluid channels **752**. The integrated manifolds **716** are formed from a primary channel **771** that runs substantially normal to the fluid channels **752** with a port **772** along the length of the substrate to which the fluidics shown in FIG. **7**A can couple. The fluid channels **752** couple directly into the primary channel **771**.

FIG. **7**D shows a plan view of an example substrate suitable for use as the second substrate **706** of the cell transduction stack **702** shown in FIG. **7**A. As the fluid channels **752** of the second substrate are designed to carry cells and genetic information introduction agents or other additives that are often sensitive to high levels of shear and/or high shear gradients, the second substrate **706** includes integrated manifolds **716** with more complex geometries than those included in the substrates forming the first and third substrates **704** and **706** of the cell transduction stack **702**. The manifolds shown in FIG. **7**D include primary channels **780** that run substantially normal to the fluid channels **752** formed in the substrate. The primary channels **780** end, at one end, at a fluid port **782** along the length of the substrate, and to which the fluidics of the cell transduction system **700** shown in FIG. **7**A can couple. The primary channels **780** narrow as they near their ends furthers from the fluid ports **782**, as secondary channels **784** branch off from the primary channel **780**. The second channels **784** then bifurcate into tertiary and quarternary channels **786** and **788**, until they finally bifurcate into the fluid channels **752** of the substrate. Further details for such a manifold can be found in U.S. Pat. No. 9,421,315, the entirety of which is incorporated by reference.

Experimental Results

A cell transduction system having the configuration shown in FIGS. **7**A-**7**D was tested in comparison to traditional spinoculation transduction techniques to evaluate the transduction efficiency of the system. FIG. **8** shows the results of the experiments.

In the experiment, one million cells derived from an immortalized human T-lymphocyte cell-line (Jurkat cells), which were suspended in cell media, were introduced into the device of FIGS. **7**A-**7**D along the sample loading pathway shown in FIG. **7**A. The cells were entrained within the cell entrainment cavities **748** by flowing media (RPMI, supplemented with 10% fetal bovine serum (FBS) and antibiotics (P/S)) containing a commercial lentiviral vector encoding green fluorescent protein (GFP) with a vertical flow rate maintained at 0.1 ml/min for 30 min. The vertical flow passed through the cell transduction system **700** along the cell entrainment pathway shown in FIG. **7**A. The cells were then washed in the device for 10 minutes with a reverse vertical flow (at a flow rate of 0.1 ml/min) using fresh media. For control samples, one million Jurkat cells were spinoculated (e.g., centrifugally inoculated) at **800**G for 30 min with commercially-available lentiviral vector encoding GFP, in accordance with a standard protocol for spinoculation of lentiviral vectors. The multiplicity of infection (MOI) in both instances was 3.0. Negative control samples were incubated without a vector.

Following treatment with the GFP+ lentiviral vector, cells were removed from the device (in the case of experimental samples) or the spinoculation tubes (in the case of control samples). The cells were re-suspended in fresh RPMI media and cultured for four days under standard cell culture conditions. The cultured cells were analyzed using flow cytometry using forward and side scatter to assess efficiency of gene transduction in cells. The results are expressed in FIG. **8** as percentages of lentiviral-tranduced GFP+ cells in the total population of viable cells.

As shown in FIG. **8**, the devices of the disclosure are useful in the transduction of exogenous genetic material using suitable vectors, such as, for example, lentiviral vectors. For instance, Jurkat cells treated with the lentiviral vectors in the devices of the disclosure were stably or transiently transduced with the viral construct and the transduced cells expressed measurable quantities of the marker protein (GFP), which expression was maintained for at least 4 days post-treatment with the viral vector. Furthermore, the expression of the marker protein was specific to virally-transduced cells, as control cells expressed negligible amount of the marker protein. The results further show that the devices are effective in transducing suspended cells, which are generally more difficult to transduce compared to adherent cells.

More importantly, the results demonstrate that the devices of the disclosure confer greater transduction efficiency compared to spinoculation methods. As shown in FIG. **8**, about 45% of the cells were transduced with the lentiviral vector using the device of the instant disclosure. In comparison, the transduction efficiency achieved with a routine spinoculation procedure was appreciably lower, at about 23%. The results demonstrate that the devices of the disclosure confer a significant improvement (about 100%) in the overall transduction rates compared to spinoculation methods. Accordingly, the results show that the devices of the disclosure confer significant advantages over existing systems and methods for gene delivery into target cells. It should be further noted that the devices of the disclosure improve transduction efficiency without compromising cell viability.

While this specification contains many specific implementation details, these should not be construed as limitations on the scope of any inventions or of what may be claimed, but rather as descriptions of features specific to particular implementations of particular inventions. Certain features that are described in this specification, in the context of separate implementations, can also be implemented in combination in a single implementation. Conversely, various features that are described in the context of a single implementation can also be implemented in multiple implementations separately or in any suitable sub-combination. Moreover, although features may be described above as acting in certain combinations and even initially claimed as such, one or more features from a claimed combination can in some cases be excised from the combination, and the claimed combination may be directed to a sub-combination or variation of a sub-combination.

Similarly, while operations are depicted in the drawings in a particular order, this should not be understood as requiring that such operations be performed in the particular order shown or in sequential order, or that all illustrated operations be performed, to achieve desirable results. In certain circumstances, multitasking and parallel processing may be advantageous. Moreover, the separation of various system components in the implementations described above should not be understood as requiring such separation in all implementations, and it should be understood that the described components and systems can generally be integrated together in a single product or packaged into multiple products.

References to "or" may be construed as inclusive so that any terms described using "or" may indicate any of a single,

more than one, and all of the described terms. The labels "first," "second," "third," and so forth are not necessarily meant to indicate an ordering and are generally used merely to distinguish between like or similar items or elements. Thus, particular implementations of the subject matter have been described. Other implementations are within the scope of the following claims. In some cases, the actions recited in the claims can be performed in a different order and still achieve desirable results. In addition, the processes depicted in the accompanying figures do not necessarily require the particular order shown, or sequential order, to achieve desirable results.

What is claimed is:

1. An apparatus comprising:

a first substrate defining at least one first flow chamber coupled to a first fluid manifold;

a second substrate defining a cell entrainment layer, the cell entrainment layer including:

at least one second flow chamber;

a plurality of cell entrainment cavities, wherein each of the cell entrainment cavities opens at one end into one of the at least one second flow chambers, extends through the second substrate, and is sized to hold at least one cell;

at least one inlet to the at least one second flow chamber substantially within the plane of the second substrate; and

at least one outlet from the at least one second flow chamber substantially within the plane of the second substrate;

a first membrane positioned between the first substrate and the second substrate, the first membrane includes a plurality of pores that are small enough to prevent the passage of cells and large enough to allow the passage of a virus;

a third substrate defining at least one third flow chamber coupled to a second fluid manifold; and

a second membrane positioned between the second substrate and the third substrate, the membrane includes a second plurality of pores that are small enough to prevent the passage of viral particles but large enough to allow the passage of cell media.

2. The apparatus of claim **1**, wherein the at least one first flow chamber, the at least one second flow chamber, and/or the at least one third flow chamber comprise a respective substantially planar flow field that couples to a corresponding manifold via a plurality of fluid connections.

3. The apparatus of claim **1**, wherein the at least one first flow chamber, the at least one second flow chamber, and/or the at least one third flow chamber comprise a plurality of flow channels, wherein each flow channel couples to a manifold via a single fluid connection.

4. The apparatus of claim **1**, further comprising the first and second fluid manifolds, wherein:

a first end of the first fluid manifold couples to the at least one first fluid chamber defined by the first substrate;

a first end of the second fluid manifold couples to the at least one third fluid chamber defined by the third substrate; and

a second end of the first fluid manifold is fluidically coupled to a second end of the second fluid manifold such that fluid can circulate through the first fluid manifold, the first membrane, the plurality of cell entrainment cavities, the second membrane, the second fluid manifold and back to the first fluid manifold.

5. The apparatus of claim **4**, wherein at least one of the first fluid manifold and the second fluid manifold comprises a vertical flow manifold.

6. The apparatus of claim **4**, wherein at least one of the first fluid manifold and the second fluid manifold comprises a horizontal flow manifold.

7. The apparatus of claim **4**, further comprising a waste channel coupled between the second end of the first fluid manifold and the second fluid manifold by a valve, wherein the valve is configured to selectively divert fluid flow directed out of the second end of the first fluid manifold to a waste reservoir.

8. The apparatus of claim **4**, further comprising a first pump configured to pump fluid into the second end of the first fluid manifold.

9. The apparatus of claim **8**, comprising a second pump configured to pump fluid into the second end of the second fluid manifold, and wherein the second pump is the same pump as the first pump or different than the first pump.

10. The apparatus of claim **4**, wherein the first substrate further comprises an outlet coupled to a distal end of the at least one first fluid chamber.

11. The apparatus of claim **1**, wherein the cell entrainment cavities have a greater density towards a distal end of the at least one second fluid chambers than towards a proximal end of the at least one second fluid chambers.

12. A method of cell transduction comprising:

introducing cells into at least one first flow chamber;

introducing genetic information introduction agents into the first flow chamber;

flowing a first fluid in a first direction substantially normal to the at least one first flow chamber and through a plurality of cell entrainment cavities distributed along the at least one first flow chamber having proximal ends open to respective first flow chambers, thereby entraining the introduced cells and genetic information introduction agents into the plurality of cell entrainment cavities for a first period of time, thereby allowing the genetic information carried by the genetic information introduction agents to be transduced into the entrained cells;

preventing passage, through distal ends of the cell entrainment cavities, of the cells and the genetic information introduction agents;

reversing the direction of flow of the first fluid for a second period of time, thereby releasing the cells from the cell entrainment cavities and washing the genetic information introduction agents away from the cells;

flowing the released cells out of the at least one first flow chamber for collection.

13. The method of claim **12**, wherein the at least one first flow chamber, the at least one second flow chamber, and/or the at least one third flow chamber comprise a respective substantially planar flow field that couples to a corresponding manifold via a plurality of fluid connections.

14. The method of claim **12**, wherein the at least one first flow chamber, the at least one second flow chamber, and/or the at least one third flow chamber comprise a plurality of flow channels, wherein each flow channel couples to a manifold via a single fluid connection.

15. The method of claim **12**, wherein flowing the first fluid in the first direction comprises flowing the first fluid through a first membrane having pores sized to prevent passage of the cells but large enough to allow passage of the genetic information introduction agents.

16. The method of claim **15**, wherein flowing the first fluid in the first direction further comprises flowing the first fluid

through the distal end of the cell entrainment cavities through a second membrane having pores sized large enough to allow passage of first fluid and small enough to prevent passage of the genetic information introduction agents through the second membrane.

17. The method of claim **12**, wherein flowing the first fluid in the first direction further comprises creating a circulating flow in which fluid flowing through the second membrane is redirected back through the first membrane in the first direction.

18. The method of claim **12**, wherein the genetic information introduction agents comprise viruses.

19. The method of claim **12**, wherein the cells and the genetic information introduction agents are introduced into the first flow field substantially simultaneously.

20. The method of claim **12**, wherein the cells are introduced into the first flow field prior to the introduction of the genetic information introduction agents into the first flow field.

21. An apparatus comprising:

a first substrate defining at least one first flow chamber coupled to a first fluid manifold;

a second substrate defining at least one second flow chamber comprising:

a first membrane positioned between the first substrate and the second substrate, wherein

the first membrane includes a plurality of pores that are small enough to prevent the

passage of cells and large enough to allow the passage of a virus;

a third substrate defining a third flow chamber and coupled to a second fluid manifold;

a second membrane positioned between the second substrate and the third substrate, the membrane includes a second plurality of pores that are small enough to prevent the passage of viral particles but large enough to allow the passage of cell media;

and a means for entraining cells within the at least one second flow chamber as a result of a flow of fluid across the first and second membranes.

22. The apparatus of claim **21**, wherein the means for entraining cells comprises the second membrane.

23. The apparatus of claim **21**, wherein the second membrane comprises one of a patterned membrane and an unpatterned membrane.

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